Mayo Clinic Proceedings

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Bypass Surgery for Vascular Disease of the Carotid System

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A series of 58 operations on 56 patients, in whom a branch of the superficial temporal artery was anastomosed to a branch of the middle cerebral artery (STA-MCA bypass or Yaşargil procedure), is reviewed. These operations were performed chiefly for occlusions or for inaccessible stenotic lesions of the internal carotid or middle cerebral arteries. Patency in eight patients operated on from April 1971 through November 1973 was low (25%). Patency in patients operated on since July 1974 has been high (95%). There have been no deaths and no major ischemic strokes attributable to the surgery. The rationale for this procedure is considered in relationship to the anatomy and physiology of the cerebral circulation and the pathogenesis of syndromes of cerebral ischemia. The operation appears to have a low morbidity in good-risk patients. The role of this operation in managing common manifestations of cerebral vascular disease such as focal transient cerebral ischemic attacks (TIAs) and amaurosis fugax, although not fully established, appears encouraging. The procedure seems useful for orthostatic cerebral ischemia caused by multiple occlusions of major extracranial (and intracranial) vessels and, occasionally, for progressing strokes related to internal carotid artery occlusion, both of which are relatively uncommon manifestations of cerebral vascular occlusive disease. It may have application in the rare "slow stroke." The procedure is probably of limited value, if any, in the management of large completed infarcts but may be indicated in selected patients with small infarctions who have preserved most of their cerebral function and who have had evidence of subsequent focal ischemic events. The procedure is useful for bypassing giant aneurysms or basofrontal tumors invading major vessels. It may have a role in the management of fibromuscular disease of the internal carotid artery.

Yaşargil and associates,[1,2] after extensive laboratory work with Donaghy,[3] performed the first anastomosis of the superficial temporal artery to a branch of the middle cerebral artery. Since that date, almost a decade ago, this operation has been used with varying frequency in a number of centers throughout the world.[4-7]

The purpose of this report is to evaluate the indications for this procedure and to discuss the results and complications noted in our series. We will consider the rationale for the operation and attempt to place in some perspective its possible role in the management of occlusive vascular disease of the carotid system. The surgical technique is essentially that described by Yaşargil and associates[1,2] with minimal modifications.[8] Typical and unusual cases will be described to illustrate the types of patients operated on and the complications encountered.

This investigation was supported in part by Research Grant NS-6663 from the National Institutes of Health, Public Health Service.

NEUROLOGIC EVALUATION

All patients had a detailed neurologic examination by a member of our Department of Neurology at the time of admission to the hospital. Thereafter, they were evaluated daily during their hospital course. Those patients who did not return for follow-up examination after dismissal were contacted by telephone to determine their current clinical condition.

Retinal artery pressures (RAPs) were obtained routinely before and after surgery. These measurements were repeated during the follow-up examination. Changes in RAP served to indicate the relative increase in perfusion pressure to the carotid system from a patent bypass graft. These data will be reported elsewhere.[9]

Patients were categorized clinically according to the following defined symptom complexes.

Transient Ischemic Attacks. Retinal (Amaurosis Fugax).—Transient monocular blindness usually lasts less than 1 hour and most commonly lasts only 3 to 5 minutes. The onset is rapid, often described as a shade coming down slowly over one eye. A positive scotoma is on occasion reported preceding the blindness.

Cerebral.—A transient focal neurologic deficit of less than 24 hours' duration is considered to represent a transient cerebral ischemic attack (TIA). Carotid system TIAs are most commonly manifested by transient weakness or numbness in the hand area and, when the dominant hemisphere is involved, by dysphasia. Severe TIAs produce a transient hemiparesis. Localization of a focal deficit to the foot alone is a most uncommon form of TIA.

Cerebral Infarction.—A neurologic deficit persisting longer than 24 hours is considered to result from a cerebral infarction. Infarction denotes death of tissue. Infarction usually produces a permanent deficit but small infarctions can result in a reversible deficit, which has been referred to by some as a reversible ischemic neurologic deficit (RIND). This term was not used as a descriptive term in this group.

Progressing Stroke.—This is a progressive stepwise neurologic dysfunction evolving over a period of hours or days. The temporal profile is characteristic; the dysfunction begins as a relatively minor deficit that culminates in a major deficit in the absence of treatment.

Slow Stroke.—This is a most uncommon entity in which the temporal profile is slow, often over a period of weeks, and not stepwise as in a progressing stroke. The history often suggests the presence of a mass. The focal deficit often is most severe in the foot, a symptom related to ischemia in the watershed zone of cerebral perfusion.

Primary Orthostatic Cerebral Ischemia.—This term describes generalized, nonfocal, cerebral hypotension related to erect position in a patient with multiple occlusions of major extracranial vessels. The patient complains, on standing up, of light-headedness, a fainting sensation, or actual syncope. This must be distinguished from systemic orthostatic hypotension in which the systemic blood pressure decreases when the patient rises. Difficulty in walking, unsteadiness of gait, and dimness in vision may occur transiently. RAPs have an orthostatic decrease disproportionate to that of the systemic pressure. Episodes of focal ischemia, transient or culminating in infarction, may also occur. Although the family may report changes in behavior or memory, this group of patients is different and separate from those suffering from senile dementia.

Ischemic Pain.—Pain referred to the eye or temporal area is sometimes present in patients suffering from a high-grade stenosis or occlusion of the internal carotid artery. Typically this pain is worse with erect posture. The mechanisms for this type of pain will be considered elsewhere.[10]

VASCULAR EVALUATION

Angiograms were performed before surgery in all patients. The patency of the anastomosis was evaluated in the immediate (24 hours) postoperative course by an ultrasound blood-flow detector. If doubt existed about patency, an angiogram was done at this time. However, angiograms were, in most cases, done at the time of follow-up examination. In those patients in whom a return visit was unlikely because of travel distance or other reasons, an angiogram was done before dismissal.

The ultrasound blood-flow detector is a highly reliable, noninvasive means of examining blood flow through the anastomosis. When used over the site of anastomosis, this probe identifies two sounds. One represents a background hum, likely originating from the general cranial circulation; it is not altered by digital occlusion of the superficial temporal artery. The other sound arises from blood flow through the anastomosis and is obliterated by pressure over the parent superficial temporal artery proximal to the site of anastomosis. This latter sound has characteristic systolic and diastolic phases and is higher pitched and louder than the former; it has many qualities similar to the bruits heard with a stethoscope over large arteries at sites of turbulent flow.

Table 1.—**Early Experience With Bypass Procedures, 1971 to 1973**

Case	Age; sex	Date	Vascular pathology*	Clinical symptoms	Anastomosis patent	Clinical result	Comment
1	51; M	4/19/71	Bilateral ICA occlusions	Orthostatic cerebral ischemia	No	No change	Memory and intellectual impairment
2	56; M	4/24/72	MCA stenosis	Progressing stroke with infarction	No	Poor	Progressive deficit on anticoagulants; hemiplegia at time of surgery
3	52; F	5/11/73	Giant aneurysm	Headache; lesion of cranial nerve VI	No	Excellent	Had previous common carotid ligation; ICA ligated after STA-MCA* anastomosis
4	44; M	7/1/73	Bilateral ICA occlusions	Orthostatic cerebral ischemia	No	Poor	. . .
5	64; M	7/26/73	ICA occlusion	Progressing stroke	Yes	Excellent	Focal deficit primarily in lower extremity; residual deficit in foot
6	62; M	10/3/73	Bilateral ICA occlusions	Orthostatic cerebral ischemia; dysphasia	Yes	Excellent	Major improvement in speech; RAPs increased postoperatively
7	46; M	11/8/73	Fibromuscular disease	Small infarction; frequent TIAs	No	Excellent	TIAs related to position, speech, and arm; unilateral headache
8	60; M	11/27/73	Ipsilateral ICA occlusion; contralateral ICA siphon stenosis	Orthostatic cerebral ischemia; old infarction	No	No change	Focal deficit primarily in lower extremity

*ICA = internal carotid artery; MCA = middle cerebral artery; STA = superficial temporal artery.

Blood flow was determined with the use of a Doppler velocity flow probe and the postoperative angiogram. The volume of flow was computed from the velocity of the flow and the diameter, and hence area, of the artery at the point of velocity analysis. Technical considerations and results of this measurement will be considered elsewhere.[11]

CASE MATERIAL

Preliminary Experience (January 1971 to January 1974).—Patients in this group, in general, presented with urgent or semi-urgent problems and were often operated on as emergencies, the surgery representing a desperate effort. The cases are summarized in Table 1.

Recent Experience (July 1974 to July 1976).—Patients in this group were usually operated on as elective cases. This series was begun after further laboratory work to perfect microsuture techniques.[8]

RESULTS

Graft Patency.—Patency in the early series was 25%. Patency in the recent experience was 95%. Causes for early graft failure included trauma to vessels at the site of anastomosis, poor coaptation of endothelial surfaces along the suture line, small superficial temporal artery branch (less than 1.0 mm OD), small recipient cortical vessel (less than 1.0 mm OD), and damage to the superficial temporal artery as it was dissected from the scalp. The reversal of the initial pathologic process in the internal carotid artery, leading to a marked reduction in flow through the graft, resulted in a late (over 6 weeks) occlusion in one case.

Arterial Pathology.—The arterial pathology in these 58 cases included: internal carotid occlusion (35), fibromuscular disease (7), middle cerebral artery stenosis (5), carotid siphon stenosis (5), aneurysm (4), Moya-Moya (1), and invasion of vessel wall by tumor (1).

Clinical Symptomatology.—An evaluation of the individual patient's general medical and neurologic condition at the time of follow-up examination is included in the data of Tables 1 and 2. However, these patients often were suffering from more than one symptom complex of cerebral ischemia. Therefore, it is necessary to consider the apparent result of the operative procedure according to the clinical categories used for analysis (the number of symptom complexes exceeds the number of patients operated on).

Transient Ischemic Attacks.—1. Amaurosis fugax: present in four cases; there was no recurrence of symptoms following successful surgery in this group.

Table 2.—**Experience With Bypass Procedures, 1974 to 1976**

Case	Age; sex	Date	Vascular pathology*	Clinical symptoms	Anastomosis patent	Clinical result	Comment
9	61; M	7/9/74	Bilateral ICA occlusions	TIAs; orthostatic cerebral ischemia	Yes	Good	See case reports
10	36; M	7/19/74	MCA stenosis	TIAs	Yes	Excellent	Maintained on long-term anticoagulants
11	46; F	8/20/74	ICA occlusion	Slow stroke; infarction	Yes	Fair	At surgery major conducting vessels were occluded; RAPs increased postoperatively
12†	56; M	10/14/74	Bilateral ICA occlusions	Progressing stroke; orthostatic cerebral ischemia	Yes	Good	Deficit most prominent in lower extremity; hand and speech also involved
13	54; M	10/18/74	MCA stenosis; carotid stenosis	Infarction	Yes	Fair	Improvement no different from that expected from infarction without treatment
14	58; F	10/30/74	Bilateral siphon stenosis	Infarction; TIAs	Yes	Excellent	Bilateral stenotic lesions progressed to occlusions during follow-up
15	77; F	11/13/74	Giant aneurysm	Ophthalmoplegia; retro-orbital pain	No	Excellent	Subsequent ICA ligation
16	60; M	11/21/74	ICA occlusion	Amaurosis fugax; TIAs	Yes	Excellent	TIA in postoperative period
17	73; M	12/2/74	ICA occlusion	TIAs	Yes	Excellent	Two to three TIAs per week while on anticoagulants preoperatively; hand, foot, and speech affected
18	45; F	12/27/74	ICA occlusion	Amaurosis fugax; TIAs	Yes	Excellent	TIAs persisted on anticoagulants (poorly controlled) before surgery
19†	56; M	1/13/75	Bilateral ICA occlusions	Orthostatic cerebral ischemia	Yes	Good	. . .
20	50; F	3/10/75	ICA occlusion	Amaurosis fugax; TIAs	Yes	Excellent	Ischemic symptoms atypical, localized to lower extremity; RAPs increased postoperatively
21‡	42; M	3/24/75	ICA fibromuscular disease	TIAs; headache	Yes	Excellent	Bypass grafts patent, previous fibromuscular disease no longer apparent; bilateral procedures
22‡	42; M	5/13/75	ICA fibromuscular disease	. . .	Yes	Excellent	. . .
23	43; M	6/9/75	ICA occlusion	Amaurosis fugax; venous-stasis retinopathy	Yes	Excellent	See case reports
24	54; F	6/17/75	Giant aneurysm	SAH; infarct	Yes	Excellent	See case reports
25	65; M	6/19/75	ICA occlusion	Slow stroke	Yes	Fair	Ambulatory; no progression of paresis but no improvement
26	69; M	6/27/75	ICA occlusion	TIAs	Yes	Excellent	No further TIAs
27	55; F	7/8/75	ICA fibromuscular disease	TIAs; headache	Yes	Excellent	. . .
28	65; M	7/14/75	ICA occlusion	Slow stroke	Yes	Good	See case reports
29	44; F	8/11/75	ICA siphon stenosis	TIAs	Yes	Excellent	Transient ischemic symptoms in foot area 1 week postoperatively
30	57; M	8/19/75	Bilateral ICA occlusions	Orthostatic cerebral ischemia	Yes	Good	See case reports
31	40; F	8/21/75	ICA fibromuscular disease	TIAs	Yes	Excellent	. . .

*ICA = internal carotid artery; MCA = middle cerebral artery; SAH = subarachnoid hemorrhage.
†Same patient.
‡Same patient.

Table 2.—*Continued*

Case	Age; sex	Date	Vascular pathology*	Clinical symptoms	Anastomosis patent	Clinical result	Comment
32	6; F	9/3/75	Moya-Moya	Slow stroke; TIAs	Yes	Good	Slow improvement in the previously progressing paresis
33	48; M	9/19/75	Siphon stenosis	Slow stroke	Yes	Fair	Stable; little improvement
34	60; M	11/10/75	Bilateral ICA occlusions	Slow stroke	Yes	Good	. . .
35	44; M	11/12/75	Siphon stenosis	TIA	Yes	Good	Remains on anticoagulants
36	67; M	11/18/75	Bilateral ICA occlusions	Orthostatic cerebral ischemia; progressing stroke; infarction	Yes	Good	Aphasia, right hemiparesis improved
37	75; M	11/28/75	Bilateral ICA occlusions	Orthostatic cerebral ischemia; infarction	Yes	Fair	Speech unchanged; other symptoms improved
38	47; F	12/30/75	Fibromuscular disease	TIAs	No	Excellent	Apparent reversal of fibromuscular disease process on repeat angiograms
39	55; M	1/2/76	ICA occlusion	Infarction; TIAs	Yes	Excellent	. . .
40	66; M	1/8/76	Right ICA occlusion	TIAs	Yes	Excellent	See case reports
41	57; M	1/22/76	ICA occlusion	Ischemic eye pain	Yes	Excellent	Eye pain subsided immediately postoperatively
42	37; F	2/25/76	Basofrontal meningioma	Orthostatic cerebral ischemia	Yes	Excellent	Tumor invading vessel wall
43	52; M	3/8/76	ICA occlusion	Small infarct	Yes	Excellent	Patient under observation with known ICA occlusion 1 year before infarction and subsequent bypass surgery
44	72; M	3/15/76	ICA occlusion	TIAs	Yes	Excellent	See case reports
45	61; M	3/26/76	ICA occlusion	Orthostatic cerebral ischemia	Yes	Excellent	. . .
46	69; M	3/30/76	MCA stenosis	Progressing stroke	Yes	Fair	Progression arrested
47	52; F	4/9/76	ICA occlusion	Slow stroke	Yes	Good	. . .
48	73; M	4/21/76	ICA occlusion	TIAs	Yes	Excellent	TIAs persisting on anticoagulants
49	32; F	4/16/76	ICA occlusion	TIAs	Yes	Excellent	TIAs persisting on anticoagulants
50	60; M	5/18/76	ICA occlusion	TIAs	Yes	Good	. . .
51	56; M	6/9/76	MCA stenosis	TIAs; small infarct	Yes	Excellent	. . .
52	59; F	7/1/76	ICA occlusion	TIAs; slow stroke	Yes	Excellent	See case reports
53	41; F	7/8/76	ICA occlusion	TIAs; infarction	Yes	Good	Simultaneous patch graft to external carotid artery
54	5; M	7/12/76	ICA giant aneurysm	Proptosis; headache	Yes	Excellent	Simultaneous ICA ligation
55	57; M	7/16/76	Ipsilateral ICA occlusion; contralateral ICA stenosis	TIAs; slow stroke; orthostatic cerebral ischemia	Yes	Excellent	Performed endarterectomy on contralateral side 10 days before bypass graft
56	32; F	7/23/76	Bilateral ICA siphon stenosis; left ICA dissection	Small infarction and TIA	Yes	Excellent	Very small STA branches
57	40; F	7/28/76	Fibromuscular disease; ICA	TIAs; small infarction	Yes	Excellent	Coexisting fibromuscular disease of renal arteries
58	32; F	7/28/76	Occlusion of ICA at origin of and involving MCA	TIAs; small infarction	Yes	Excellent	Fibromuscular disease of opposite ICA

*ICA = internal carotid artery; MCA = middle cerebral artery; SAH = subarachnoid hemorrhage; STA = superficial temporal artery.
†Same patient.
‡Same patient.

2. Cerebral: present in 27 cases. In two patients, both suffering from apparent fibromuscular dysplasia of the internal carotid artery, the bypass graft occluded. TIAs did not return in either of these patients. The fibromuscular process progressed to occlusion in one patient but reversed to normal in the other patient. In the 25 cases in which the bypass graft remained patent, TIAs did not recur.

Cerebral Infarction.—This was present in 14 cases. A patent bypass graft did not seem to alter the rate of recovery from a completed infarction in this group. However, a recurrent or additional infarction in the appropriate hemisphere did not occur during the period of follow-up in any of these patients.

Progressing Stroke.—This was present in five cases. The graft did not remain patent in one case, in which the stroke continued to progress. In the four cases in which the graft remained patent, there was excellent recovery in one, good recovery in two, and arrest of progression in one patient who subsequently died from a myocardial infarction after dismissal from the hospital.

Slow Stroke.—This existed in nine cases. The graft remained patent in seven patients, and progression of symptoms ceased in four. Two patients had slight improvement in the preexisting deficit; one patient had early improvement but, 6 months later, deterioration occurred. No patients had dramatic improvement.

Primary Orthostatic Cerebral Ischemia.—This was present in 13 cases. The graft occluded in three patients, who remained unchanged from their preoperative condition. The graft remained patent in nine patients and major improvement occurred in each.

Ischemic Pain.—This was present in one case. Successful bypass surgery produced dramatic relief of pain in this patient.

Giant Aneurysm or Basofrontal Mass.—One of these lesions was present in five cases. The graft occluded in two patients, who thereafter tolerated ligation of the internal carotid artery without incident. The graft remained patent in three cases, becoming the major source of middle cerebral artery flow in one patient and producing dramatic relief of symptoms in the other two.

Complications.—In addition to occlusion of the graft, considered above, other complications were encountered. There were minor problems with a small portion of the scalp incisions in some of the early cases. This was avoided later by using a wider-based scalp flap. No patients had infection or major areas of tissue necrosis.

Four patients had TIAs in the postoperative period (none of these patients had been operated on for TIA). All responded well to anticoagulation. One patient (see below, case 28) developed a cerebral infarction 6 weeks after surgery—related, apparently, to temporary occlusion of the bypass graft.

One patient (see below, case 9) sustained an intracerebral hematoma in the immediate postoperative period. This was deep to the site of anastomosis and apparently arose from a ruptured parenchymal vessel.

Typical Cases. TIA and Carotid Occlusion, Case 40.—This 66-year-old retired laborer with hypertension, arteriosclerotic heart disease, and occlusive peripheral vascular disease was seen in January 1976 after experiencing five episodes of transient numbness involving the left arm. The first occurred in February 1975, the others just before evaluation at this clinic. These episodes were believed to represent TIAs, although they had failed to respond to treatment with warfarin. A right carotid bruit had been heard in the past but, on examination here, was not present. RAPs were significantly reduced on the right, being recorded as 79/33 compared to 127/55 on the left. There was no neurologic deficit. Angiography showed occlusion of the right internal carotid artery just distal to its origin and moderate atheromatous change involving the left carotid siphon. Intracranially, the right anterior cerebral artery filled on cross-fill from the left while the right middle cerebral and posterior cerebral arterial groups filled via collaterals through the right ophthalmic artery. A right superficial temporal-to-middle cerebral branch anastomosis was done, using a posterior branch of the superficial temporal artery.

The postoperative course was uncomplicated. Angiograms done 1 week postoperatively showed good flow through the anastomosis into the middle cerebral complex, with the superficial temporal artery being increased to 2 mm in diameter from 1 mm preoperatively (Fig. 1). Flow through the anastomosis was calculated at 94 ml/min. The patient has remained asymptomatic over a 4-month follow-up.

TIA and Carotid Occlusion, Case 44.—This 72-year-old retired maintenance employee returned to the Mayo Clinic because of TIAs involving the left upper extremity. He had had left carotid endarterectomy in 1974 for a high-grade stenosis of the left internal carotid artery. At that time, a high-grade stenosis was also identified in the right internal carotid artery. A right carotid endarterectomy had been recommended to the patient on the basis of the angiographic appearance of the lesion. The patient was asymptomatic at that time and he elected not to have right carotid surgery. At the time of dismissal from the hospital in August 1974, a systolic-diastolic bruit was audible over the bifurcation of the right common carotid artery. Preoperative RAPs of 15/8 bilaterally had improved after endarterectomy to 52/24 on the right and 81/14 on the left.

The patient returned here in March 1976, with a chief complaint of episodes of weakness of the left arm, noted particularly in the morning after ambulation. He also reported some unsteadiness on his feet at this time.
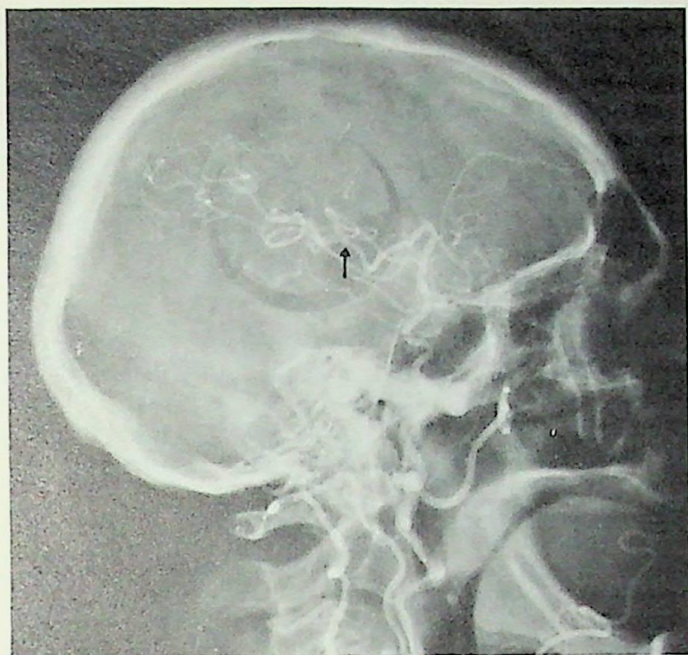
Fig. 1 (case 40). Postoperative angiogram 1 week after surgery. The diameter of the superficial temporal artery increased from 1 mm before surgery to 2 mm postoperatively. *Arrow* indicates site of anastomosis.

These complaints were present daily. Physical examination revealed mild decrease in the alternate-motion rate in the left hand. The bruit present over the right carotid artery at the time of dismissal from the hospital in 1974 was no longer audible. RAPs at this time were 59/10 on the right and 89/23 on the left.

Angiography demonstrated occlusion of the right internal carotid artery. At bypass surgery, a right superficial temporal artery was anastomosed to a temporal branch of the middle cerebral artery. The patient had an uncomplicated recovery and has had no recurrence of his preoperative symptoms. He was dismissed from the hospital 10 days after the operative procedure. Postoperative angiograms demonstrated a patent graft (Fig. 2).

Orthostatic Cerebral Ischemia, Case 30.—This 57-year-old man was admitted to the hospital with a history of ataxia and of increasing impairment in memory; he often had attacks of syncope. His brachial blood pressure was 170/80 mm Hg on lying down and 140/80 mm Hg on standing. Angiography revealed occlusion of both internal carotid arteries and a marked stenosis of the left external carotid artery. Endarterectomy of the left external carotid artery was attempted initially. This procedure was unsuccessful because the artery was diffusely involved with atherosclerosis and was occluded postoperatively from an intimal flap. Thereafter, the patient was unable to sit up in bed without losing consciousness. RAPs were 23/13 on the left and 11/0 on the right.

Accordingly, a right internal carotid bypass procedure was performed and the right superficial temporal artery was anastomosed to a cortical branch of the right middle cerebral artery. This was a large superficial temporal artery and the recipient cortical vessel was larger than average.

Postoperatively, this patient had a major improvement in his intellectual function. He remained depressed. He could sit and stand without fainting.

A postoperative angiogram demonstrated filling of the entire right middle cerebral arterial complex and both an-

Fig. 2 (case 44). Postoperative angiography demonstrates good filling of posterior middle cerebral complex from posterior temporal branch of right superficial temporal artery. Anterior middle cerebral complex continued to fill from collateral circulation from opposite hemisphere. Anterior projections indicated the arterial interface to lie at point of middle cerebral artery trifurcation. *Arrow* indicates site of anastomosis.

Fig. 3 (case 30). Postoperative angiography in patient with bilateral internal carotid occlusion. Most of circulation to both hemispheres was derived from markedly dilated superficial temporal artery graft (*arrows*). Preoperatively, flow was obtained from collateral circulation through ophthalmic arteries. There were no functioning channels from posterior circulation as posterior communicating arteries were very small.

Fig. 4 (case 24). Preoperative angiogram demonstrates flow of blood through partially thrombosed aneurysm and high-grade stenosis (*arrow*) of middle cerebral artery at its origin from aneurysm, possibly result of thrombotic material from aneurysm.

Fig. 5 (case 24). Repeat preoperative angiogram demonstrates lysis of clot in aneurysm but no change in stenosis (*arrow*) from presumed thrombus.

terior cerebral arteries through the right superficial temporal artery (Fig. 3). Flow analysis of this vessel indicated a blood flow of 250 ml/min. RAPs were 60/30 on the left and 40/25 on the right when last measured, 7 months after surgery. At that time, mentation was nearly normal.

Comment: This case illustrates the syndrome of orthostatic cerebral ischemia. It also demonstrates the large amounts of flow that can develop in some patients who have functioning bypass grafts. The occlusion of the external carotid artery resulted from an intimal ledge at the distal end of the endarterectomy. This is not uncommonly encountered in the external carotid artery (in contrast to the internal carotid artery) and should be considered before advising this surgery prior to superficial temporal artery-middle cerebral artery anastomosis. Subsequent to this case, we have often used a vein patch graft without endarterectomy in the external carotid artery when that vessel appeared to be diffusely involved with atherosclerosis.

Giant Aneurysm Bypass, Case 24.—This 54-year-old woman was admitted to the hospital with the chief complaint of left retro-orbital pain. Two weeks before admission, over a period of 18 hours, she developed a throbbing pain localized in the left orbital area. One week before admission, because of increasing headache and confusion, she was admitted to her local hospital, where a lumbar puncture disclosed xanthochromic spinal fluid. Angiograms showed a large aneurysm arising from the left internal carotid artery at the origin of the middle cerebral artery.

The angiogram indicated that the blood supply to the left middle cerebral artery passed through the aneurysm itself, which was either a dissecting aneurysm or an aneurysm (fusiform or berry) containing a large intraluminal clot (Fig. 4). A marked stenosis of the middle cerebral artery existed at its origin from the internal carotid artery. We believed that this aneurysm was inoperable because of the likelihood that repair of the aneurysm would effectively occlude the blood supply to the middle cerebral artery.

The patient was initially managed conservatively but, over a period of 1 week of observation, she developed a slowly enlarging right homonymous field defect. Angiograms were repeated (Fig. 5) and demonstrated lysis of the previously identified clot in the lumen of the aneurysm. The stenosis of the middle cerebral artery persisted at its origin from the internal carotid artery. The nature of this stenosis was not clear; it could have been caused by atherosclerosis or a soft blood clot.

Because the patient had small superficial temporal arteries, an occipital artery bypass procedure was attempted initially. However, a suitable cortical recipient vessel could not be found. After an appropriate delay, two branches of the superficial temporal artery were anastomosed to two intracranial branches of the middle cerebral artery. Simultaneously, a Selverstone clamp was placed on the internal carotid artery in the neck and tightened until there was a drop in the perfusion pressure distal to the clamp. Over the next 3 days, the Selverstone clamp was advanced to occlude the vessel. The patient made an uncomplicated recovery. She retained the
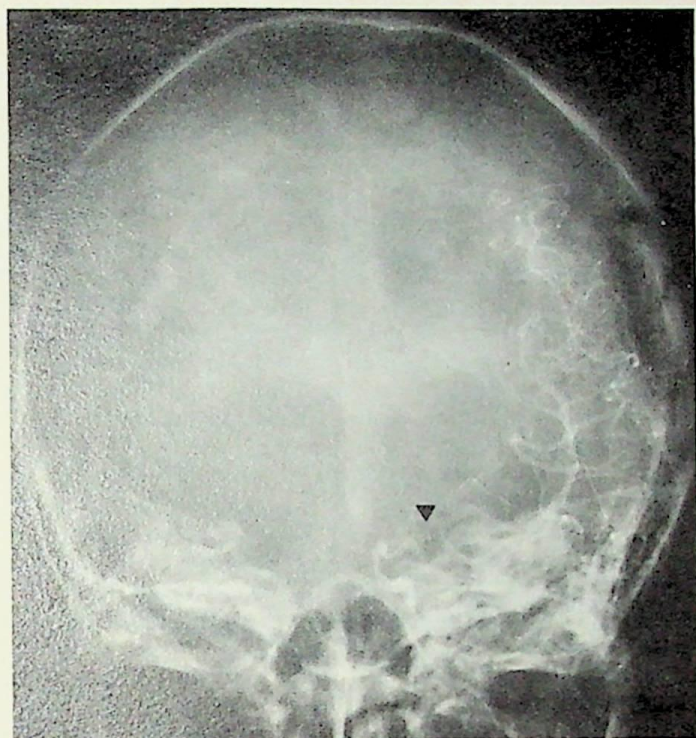
Fig. 6 (case 24). Anteroposterior film of same angiogram as in Figure 7. Aneurysm is now thrombosed and middle cerebral artery fills from superficial temporal artery-middle cerebral artery anastomosis to point of preoperative stenosis (*arrowhead*).

Fig. 7 (case 24). Postoperative angiogram demonstrates filling of left middle cerebral artery through a two-branch superficial temporal artery-middle cerebral artery anastomosis (*arrows*). Aneurysm no longer fills because internal carotid artery has been ligated. Note collateral channels in pterygoid fossa.

homonymous field defect. She was dismissed from the hospital 2 weeks after this operative procedure.

She returned to the hospital 6 months later. The neurologic examination indicated a subtotal lower quadrantanopsia. Postoperative angiography (Fig. 6 and 7) demonstrated filling of the entire middle cerebral artery through both branches of the small superficial temporal artery previously anastomosed to these vessels. There was some dilatation in the size of the superficial temporal artery. The aneurysm was no longer visible.

Comment: This case indicates the usefulness of this procedure for bypassing inoperable giant aneurysms. The source of this patient's visual field defect could have been pressure on the chiasm but the sharpness of the visual field abnormality without sloping margins indicated that it was probably in the optic radiation. Our provisional diagnosis was that the neurologic deficit had resulted from emboli through and originating from the giant aneurysm. A deficit from spasm in association with the severe middle cerebral artery stenosis could not be excluded because, at the time of the surgical procedures, blood was identified in the subarachnoid space. The preoperative angiograms had not identified a major degree of spasm.

TIA, Carotid Occlusion, and "Slow Stroke," Case 52.—This 59-year-old woman was evaluated at the Mayo Clinic in June 1976 for episodes of transient cerebral ischemia involving the entire left side of the body. These were typical TIAs and consisted of periods of weakness and numbness which usually lasted 10 to 15 minutes and then cleared without residual deficit. Although the patient had noted symptoms of this nature for about 2 years, the attacks had been occurring more frequently over the last 3 to 4 months. In addition to these TIAs, the patient complained of a slowly progressing weakness involving the left lower extremity; she walked with a slight footdrop and there was a definite decrease in the alter-
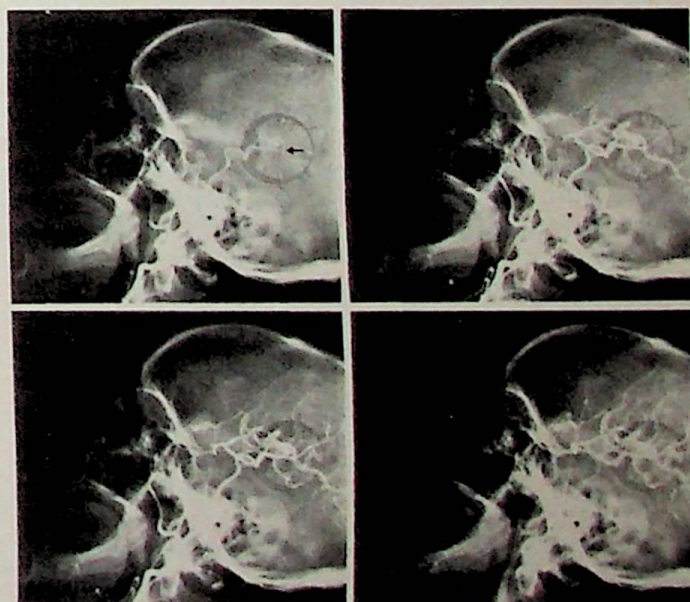
Fig. 8 (case 42). Angiograms performed before dismissal from hospital demonstrate total filling of entire middle cerebral complex through bypass graft. *Arrow* indicates site of anastomosis.
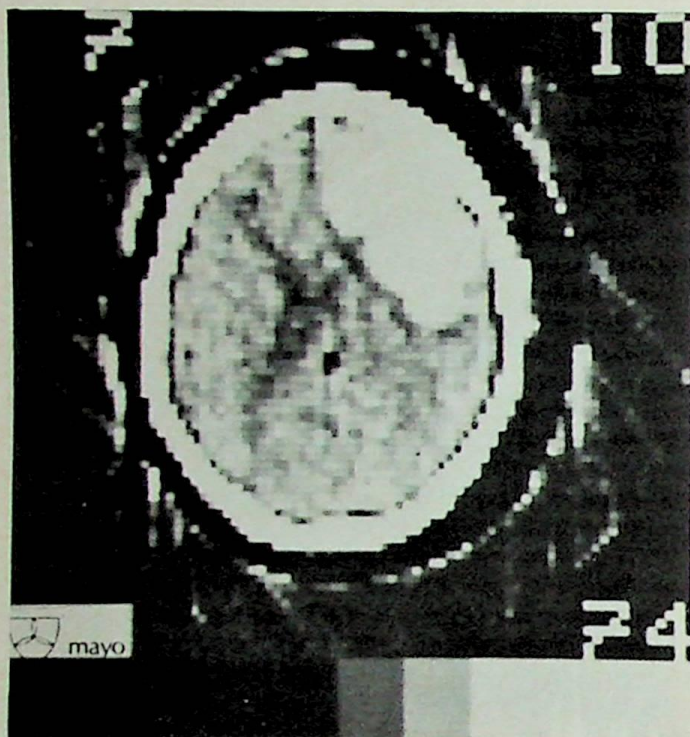
Fig. 9 (case 9). EMI scan, 14 hours after right superficial temporal artery-middle cerebral artery anastomosis, shows large intracerebral hematoma in premotor area on right.

nate motion rate in the left foot as compared to that in the right. This deficit was more pronounced with exercise and had limited the patient's activities and her walking distance, without rest, to 100 feet. RAPs were 39/13 on the right and 101/42 on the left. The clinical diagnosis was chronic right internal carotid artery occlusion, subsequently confirmed by angiography.

In July 1976, the anterior branch of the right superficial temporal artery was anastomosed to a posterior temporal branch of the middle cerebral artery. Except for a questionable TIA involving the left upper extremity, consisting of a 5-minute episode of numbness without weakness that occurred 3 days after the operative procedure, the patient made an uncomplicated recovery. By the fifth postoperative day, her exercise tolerance was markedly improved as compared to her preoperative state and it was possible for her to walk 300 to 400 feet without developing weakness in the left foot. At the time of dismissal from the hospital, alternate motion rate in the left foot was improved as compared with its state before surgery. Angiography performed before dismissal from the hospital demonstrated total filling of the entire middle cerebral complex through the bypass graft (Fig. 8). Postoperative RAPs showed a relative increase on the side of surgery, 53/15 on the right and 77/31 on the left.

Comment: This patient had the typical symptom complex of the "slow stroke." The prompt improvement in her neurologic function after bypass surgery seemingly indicates that marginally functioning neurons were present that were viable but were not physiologically normal.

Unusual Cases. Postoperative Intracerebral Hemorrhage, Case 9.—This 61-year-old man was admitted to the hospital in June 1974 with a history of many episodes of transient weakness involving the right side of the body over the 7 years before admission. These attacks resulted in a residual awkwardness in the use of the right arm. Four months before admission, the patient had several episodes of transient weakness involving the left side of the body. Over the 2 to 3 months before admission, the patient had noted light-headedness or near syncope when standing. These symptoms were relieved when he sat or lay down. His peripheral blood pressure was 150/80 mm Hg sitting; a change with position was not documented.

The major findings on neurologic examination were diminution in alternate-motion rate in all four limbs, apraxia in the right hand, a slow guarded gait, and mild difficulty in mentation. Angiograms from the referring institution demonstrated that both internal carotid arteries were occluded.

It was thought that the patient might be a candidate for a carotid bypass procedure and, accordingly, a right superficial-temporal artery-to-cortical conducting vessel anastomosis was performed.

When he awakened from the operation, the patient's blood pressure was 240/110 mm Hg; otherwise he was unchanged from his preoperative condition.

About 1 hour later he began to complain bitterly of headache and, about 2 hours thereafter, he developed a mild left hemiparesis. The operative site was reopened; the anastomosis was patent and the brain was firm or even tight against the dura but was not bulging. The patient's level of consciousness diminished 14 hours after surgery and an EMI scan (Fig. 9) demonstrated a large intracerebral hematoma in the right frontal lobe. The patient
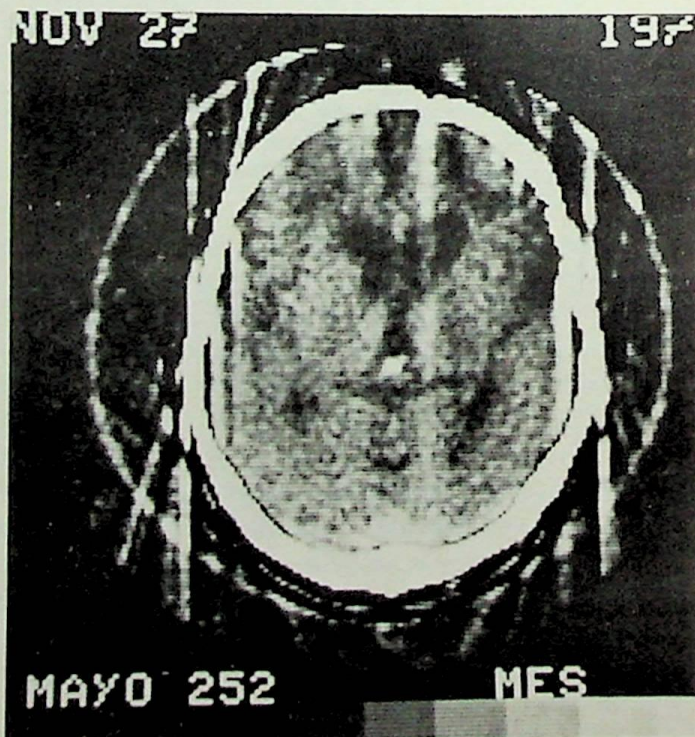
Fig. 10 (case 9). EMI scan 4 months after surgery; there is an area of radiolucency in region of old hematoma but no marked asymmetry is noted.
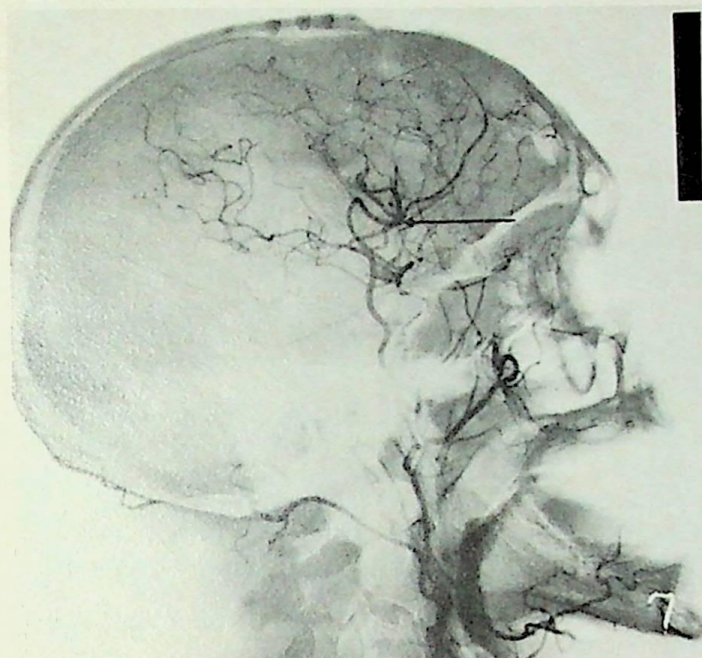
Fig. 11 (case 9). Postoperative angiogram shows filling of entire right hemispheric circulation through patent bypass graft. *Arrow* indicates site of anastomosis.

was returned to the operating room and, through a craniotomy separate from the trephine hole used for the vessel anastomosis, this hematoma was removed. The cavity of the hematoma did not connect with the site of anastomosis. The patient's neurologic function gradually improved and he was ambulatory at the time of dismissal from the hospital, about 5 weeks after the operative procedure. At that time, a repeat EMI scan displayed no residual hematoma (Fig. 10). Postoperative angiography 4 months later disclosed that the superficial temporal artery was supplying most of the cerebral circulation in the right hemisphere (Fig. 11). Examination revealed the patient's function to be essentially as described before surgery except for added mild apraxia in the left hand. The symptoms of near syncope with standing had been relieved after the surgery and (according to the patient's wife) his general intellectual function had improved.

Comment: The cause of the intracerebral hematoma in this case remains obscure. An infarction occurring just before surgery in the location of the hematoma cannot be excluded; in this type of patient, a small infarct could have appeared in this area without obvious new manifestations. The postoperative hypertension, in conjunction with a patent bypass graft, could have caused a small penetrating vessel to hemorrhage. In Tew's[12] similar case, hemorrhage developed 3 days after surgery.

Graft Occlusion With Infarction, Case 28.—This 65-year-old man was admitted to the hospital with a history of a slowly progressive right hemiparesis for 6 months. The deficit was most notable in the lower extremity, where the foot was virtually paralyzed. The patient complained of a sensation of "tightness" or stiffness involving the right limbs. His speech was normal.
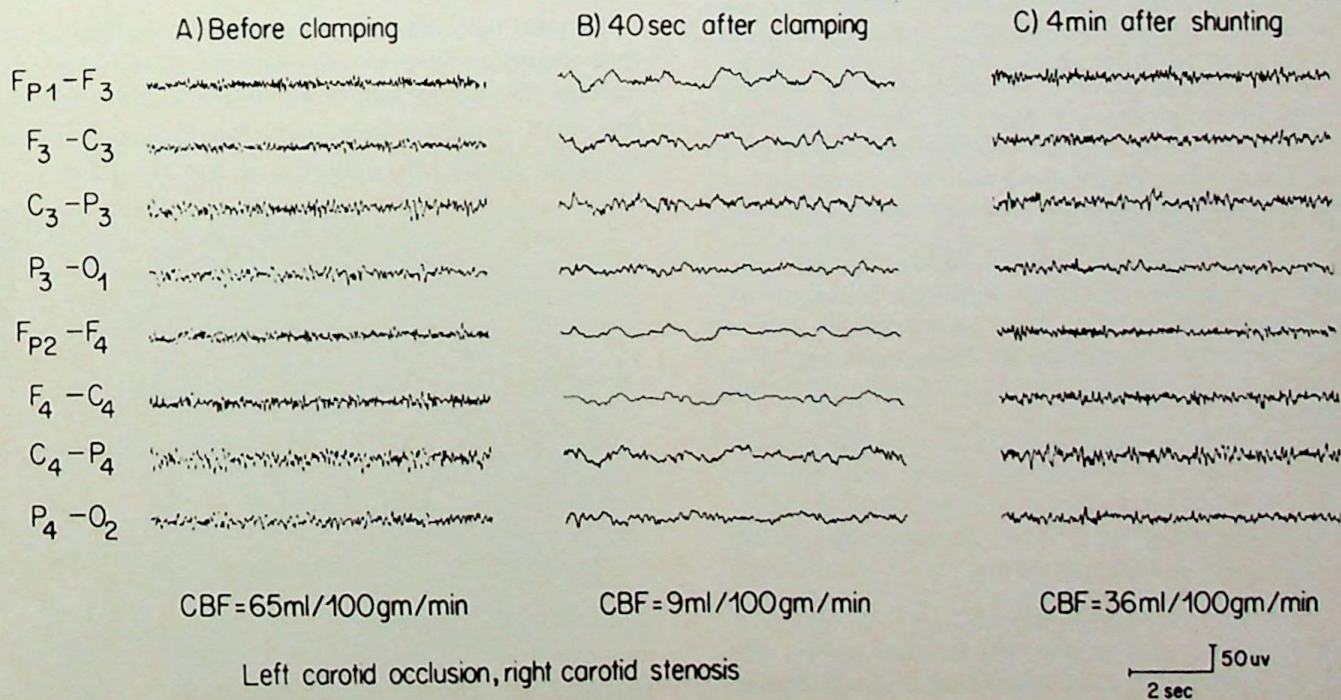
Fig. 12 (case 28). EEG tracing performed during right carotid endarterectomy shows that bilateral slowing (right greater than left) occurs after carotid occlusion (segment B), which produces decrease in cerebral blood flow in right hemisphere to 9.0 ml/100 g per minute from 65 ml/100 g per minute. This abnormality disappeared with placement of shunt (segment C). Nature of slowing suggests left hemisphere was better supplied by collaterals than right but even on left the collateral supply is inadequate when right carotid is occluded.
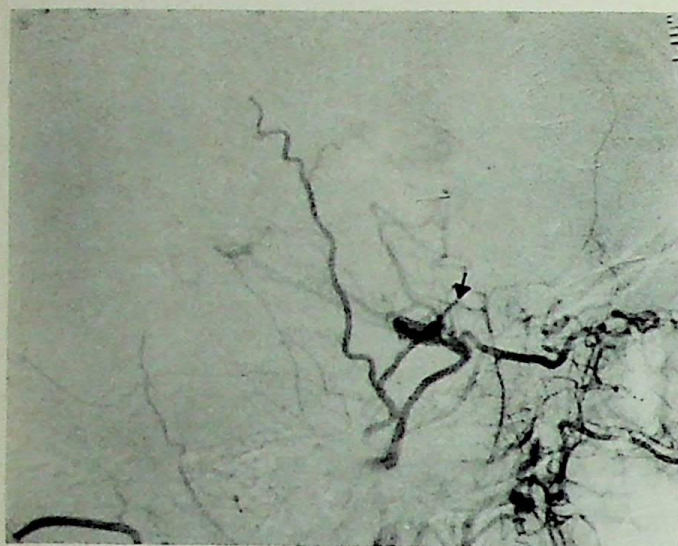
Fig. 13 (case 28). Postoperative angiogram 24 hours after surgery shows suspected occlusion of anastomosis at *arrow*.

Angiograms from the referring institution demonstrated occlusion of the left internal carotid artery and a 95% stenosis of the origin of the right internal carotid artery. The blood supply to the left cerebral hemisphere apparently was derived from collaterals through the left ophthalmic artery. RAPs were 75/45 on the right and 40/10 on the left.

We believed the patient was a candidate for a right carotid endarterectomy and a left carotid-middle cerebral bypass procedure. The right carotid endarterectomy was performed first. During the occlusion of the right carotid artery for endarterectomy, the cerebral blood flow in the right hemisphere decreased markedly from a baseline of 65 ml/100 g per minute to 9 ml/100 g per minute, and the EEG showed a rapid bilateral slowing, greater on the right than on the left (Fig. 12). Blood flow increased to 36 ml/100 g per minute after placement of a shunt, and the EEG returned to normal. A vein graft was used to repair the endarterectomized artery. The patient had an uncomplicated postoperative recovery. About 10 days after the operation, he complained of the continued presence of the abnormal subjective sensation on the right side of the body. This could not be documented by objective changes in the neurologic examination, postoperatively. RAPs were 90/55 on the right and 50/5 on the left.

A left superficial temporal artery-to-cortical branch anastomosis was performed about 3 weeks after the first procedure. The superficial temporal artery was of excellent caliber (diameter, 2 mm). It was necessary to use the angular artery itself to find a recipient vessel of sufficient size to carry the amount of flow we thought necessary in this man's case. The patient awoke from the operative procedure essentially unchanged from his state before surgery. About 2 hours later he developed some difficulty in his speech, manifested primarily in the naming of objects. The speech deficit continued during the night and by the following day the patient had marked expressive aphasia. The Doppler flow probe indicated a clicking sound at the site of anastomosis and it was our judgment that the superficial temporal artery had occluded; this was confirmed by angiography (Fig. 13).

The patient was returned to the operating room. We found that the point of occlusion was actually in the superficial temporal artery itself, where a branch of that vessel had been ligated. It was necessary to excise a 1-cm length of the distal superficial temporal artery and, accordingly, the anastomosis had to be taken down. The superficial temporal artery was again anastomosed to the angular artery and both vessels pulsated vigorously after this anastomosis. The patient awoke from surgery with marked improvement in his speech. Heparin, 3,500 U during every 4 hours, was administered postoperatively by continuous intravenous infusion.

About 48 hours after this second operative procedure the patient again developed expressive aphasia. A Doppler flow probe indicated vigorous flow through the site of the anastomosis but we decided that it was necessary to confirm this by angiography. The angiogram demonstrated filling of the entire left middle cerebral arterial complex through the anastomotic channel. Filling defects were noted which we believed were emboli rather than representations of poor washout (Fig. 14).

The heparin was discontinued when oral anticoagulation was effective. Blood flow determination indicated that the anastomosed vessel was delivering about 100 ml/min to the brain. Over the next 6 weeks his speech gradually improved, becoming essentially normal by 6 months. His referring neurologist reported an initial arrest of the progressive right-sided weakness that had been evolving over the 6 months before admission to the hospital. However, after a 4-month period of stability, a further worsening of function in the hand and foot has occurred. Speech has remained normal.

Comment: This case illustrates a number of important points. The preoperative manifestations involved the watershed area primarily, the foot being the most severely involved; the Doppler probe accurately predicted patency of the anastomosis; the
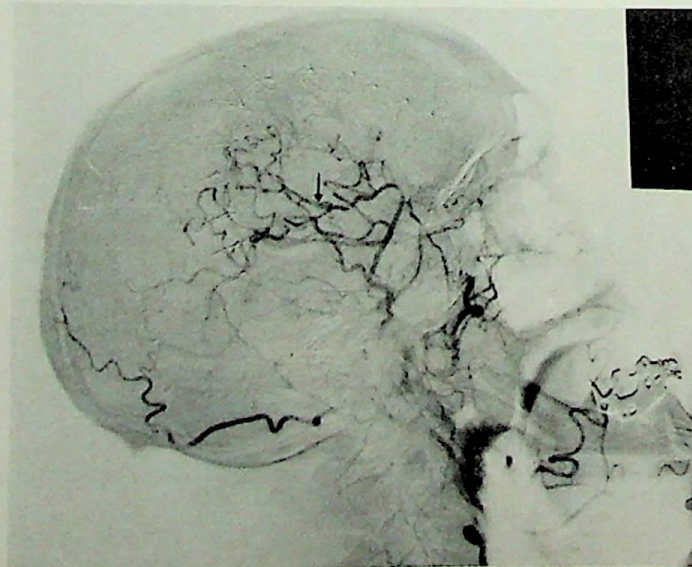
Fig. 14 (case 28). Angiogram 48 hours after reoperation shows patent bypass graft and filling of most of middle cerebral artery complex through anastomosis (*arrow*).
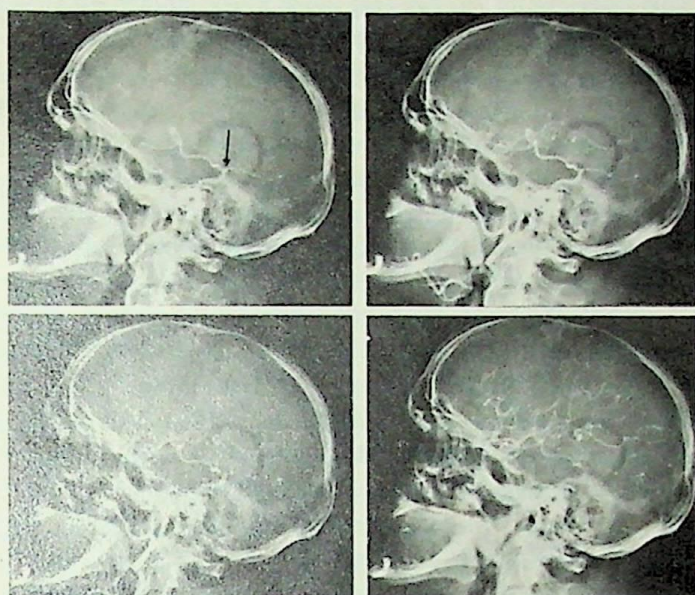
Fig. 15 (case 23). Postoperative angiogram 3 months after surgery shows filling of most of right middle cerebral artery complex through patent bypass graft. This correlated well with increase in RAP after surgery. *Arrow* indicates site of anastomosis.

ischemic deficit was reversible with subsequent restoration of flow; and embolization was possible through or from a functioning graft.

Late Infarction With Patent Bypass Graft, Case 23.—This 43-year-old man was admitted because of a gradual deterioration of visual acuity and episodes of blindness (amaurosis fugax). Funduscopic examination revealed bilateral venous-stasis retinopathy. RAPs were 18/11 on the right and 54/32 on the left. Angiograms from the referring institution showed occlusion of the right internal carotid artery.

The posterior branch of the right superficial temporal artery was anastomosed to the posterior temporal branch of the middle cerebral artery. The patient made an uncomplicated recovery and was dismissed from the hospital 10 days afterward. Before dismissal, he was instructed to have the frames of his eyeglasses readjusted, as these were noted to have produced a crease over the superficial temporal artery on the right.

About 6 weeks after dismissal, the patient suddenly developed weakness in the left lower extremity, 5 minutes after he had put on his eyeglasses in order to shave. When he was examined, we found marked weakness only in the left foot. The Doppler flow probe indicated that the anastomosis was patent. He was placed on anticoagulants and instructed to return in 3 months for postoperative angiography. The frames for his eyeglasses were readjusted to prevent pressure on the superficial temporal artery. RAPs at this time were 18/13 on the right and 66/42 on the left.

The patient was readmitted to the hospital 3 months after the operation, and angiograms done at that time demonstrated that most of the circulation of the right middle cerebral artery was derived from the superficial temporal artery (Fig. 15). The venous-stasis retinopathy had improved. RAPs were now 36/19 on the right and 84/31 on the left. The footdrop in the left lower extremity that resulted from the stroke 6 weeks after the surgery was less severe but was still present. The patient had no visual complaints.

Comment: The resolution of the venous-stasis retinopathy after an increase in RAP is not surprising; this has been reported to occur after carotid endarterectomy.[13] The development of an infarction in the watershed area, either from hypotension or from pressure on the bypass graft, is of some interest and indicates the dependency on a patent bypass graft that can develop. The delay in the elevation of the RAP probably reflects the time required for dilatation of the collateral pathways after the surgery.

DISCUSSION

In order to properly consider the results of this operation, it is necessary to review briefly some physiologic and pathologic concepts pertinent to problems of cerebral vascular disease.

Anatomic Considerations.—The circulatory system to the cerebral hemispheres is composed of two general types of arteries, conducting and penetrating.[14] The conducting vessels are the carotid, middle cerebral, anterior cerebral, vertebral, basilar, and posterior cerebral arteries plus their major (named) and minor (unnamed) branches, which form a vast network of interconnecting, anastomosing vessels on the surface of the brain. The conducting vessels may be regarded as nonresistance vessels because there is only a 10 to 15% drop in perfusion pressure from the common carotid artery to major branches of the middle cerebral artery[15] and a similar gradient from these large branches to the level of the penetrating arterioles.[16] The penetrating or regulatory arterioles enter the brain parenchyma at right angles to the surface vessels from which they are derived.[17] The system of conducting vessels serves as a pressure head or pressure equalization reservoir to provide an adequate perfusion pressure to the penetrating or nutrient vessels, wherein primary autoregulation probably resides.[16,18]

This network of conducting vessels is an ideal low-resistance vascular bed to receive a bypass graft because an increase in perfusion pressure at any point will result in a generalized increase in the pressure head of that hemisphere's arterial reservoir. The increase in perfusion pressure cannot be equated to changes in flow because of compensatory alterations that may occur in the resistance of the regulatory arterioles. However, the increased perfusion pressure does provide a functional reserve pressure

for the regulatory arterioles and may produce, in some instances, an absolute increase in flow to areas of marginal perfusion that had previously had maximal arteriolar dilatation.

Cerebral Blood Flow.—Normal blood flow to the brain approximates 50 ml/100 g per minute.[19,20] However, the brain can accommodate to a substantial reduction in flow and continue to function. There is good agreement between laboratory and clinical studies relative to the minimal amount of blood flow required to sustain normal electrical activity in the brain. Normal electrical activity cannot be equated with normal cerebral function, but the electroencephalogram has been proven to be a sensitive monitor of cerebral ischemia during carotid endarterectomy.[21] The critical level of cerebral blood flow (defined as that flow at which many patients can no longer maintain normal electrical activity) has been found to be between 15 and 18 ml/100 g per minute.[22,23]

Correlation of clinical symptoms, angiograms, and cerebral blood flow studies has indicated that blood flows of between 20 and 30 ml/100 g per minute are borderline. Patients with flow rates this low are often neurologically unstable and particularly vulnerable to the effects of emboli.[23] The development of infarction is related to both the degree and the duration of ischemia. It occurs within minutes in areas of zero flow but may take hours in regions of marginal flow.[24] Blood flow in zones of incomplete focal ischemia is nonhomogeneous with areas of reactive hyperemia, and zones of decreased perfusion often adjacent to infarcted tissue.[25,26] It is the regions of borderline flow that are theoretically benefited by a bypass procedure.

Rationale and Role for Bypass Surgery. TIA.—Retinal: The presence in the retina, in many cases, of emboli (usually cholesterol, rarely platelet aggregates) indicates that amaurosis fugax is probably embolic in origin in most instances.[27] When a carotid artery is occluded or nearly occluded in the presence of retrograde ophthalmic flow (on angiography) and a markedly lowered retinal artery pressure, the possibility exists that transient nonembolic flow alterations can occur and produce retinal ischemia.[23] Only rarely are attacks of amaurosis fugax (unilateral or bilateral) related to posture. The occurrence of amaurosis fugax in the presence of occlusion of an internal carotid artery is a possible indication for a bypass procedure.

Cerebral: The microembolic etiology of episodes of TIA was suggested by Millikan and Siekert[28] and supported by laboratory investigations by Whisnant and associates.[29] Houser and associates[23] reported that analysis of the extracranial and intracranial angiographic patterns and study of specimens removed at carotid endarterectomy supported this hypothesis. Usually, they found evidence of ulceration in patients with severe arterial stenosis. Furthermore, virtually all patients with nonobstructive plaques (<85 to 90% stenosis) had ulceration identified at operation to explain the symptoms.[23]

Although most TIAs are embolic in origin, there is evidence that some may occur as a result of systemic changes (for example, in perfusion pressure) in patients who have marginal flow from an occluded or stenosed major vessel. It is in this group of patients that bypass surgery may be particularly useful. However, the procedure is not necessarily limited to nonembolic manifestations of cerebrovascular disease. The alteration in flow patterns (for example, reversal of a retrograde ophthalmic flow pattern with possible emboli off a thrombus extending from the carotid siphon in an occluded internal carotid artery) that occur after successful bypass surgery may be important in protecting from future TIAs or infarction.[30] Furthermore, increased collateral flow to areas of marginal perfusion increases that region's tolerance to withstand small emboli without infarction.

Infarction.—Cerebral infarction, in contrast to cerebral ischemia, denotes anatomic death of tissue. Analysis of the intracranial and extracranial angiograms of patients considered for carotid endarterectomy has revealed evidence of obstruction to flow in either the intracranial or extracranial circulation in most patients with a known cerebral infarction.[23] About 50% of the patients with infarctions had angiographic evidence of a focal or internal carotid artery-middle cerebral artery slow-flow alteration indicating marginal cerebral blood flow. The cause for cerebral infarction in this group was occlusion or near obstruction of a major intracranial or extracranial conducting vessel with a reduction in the critical perfusion pressure in the pressure head reservoir. This group might be benefited by surgery to bypass an occluded major vessel.

Patients with large infarctions were not, in general, considered for bypass surgery. Patients with small infarctions were considered possible candidates for surgery if other symptoms were also present and if it appeared that the perfusion pressure could be increased to areas adjacent to the infarct zone.

Progressing Stroke.—It is necessary to analyze both intracranial and extracranial vascular patterns in discussing the causes for a progressing stroke.[23] Such an analysis reveals that, in general, these patients

are suffering from symptoms referable to multiple embolisms or high-grade extracranial obstruction to flow in conjunction with some intracranial cause for failure of the collateral circulation to provide adequate cerebral perfusion. A relatively large number of such patients have angiographic evidence of internal carotid artery-middle cerebral artery slow flow.[23] Clinically, these patients often have a progressive course that may be related to intermittent embolization in areas of marginal flow. Focal flow alterations, probably related to intracranial embolization, have been identified in about one-fourth of this group,[23] but because of the probability that emboli are lysed and are not ordinarily identified on angiography, it is assumed that they are present in a much higher percentage of cases.

The majority of such patients will improve after therapy with intravenous heparin; operative treatment in the form of endarterectomy or bypass surgery usually is not performed in the acute phase of the illness. However, a small number of patients who have a progressing stroke from a known occlusion of an internal carotid artery apparently progress because of a failure of collateral circulation, with or without superimposed emboli. It is in this small group of patients that bypass surgery seemingly has been beneficial.

"Slow Stroke."—This is a most uncommon manifestation of cerebral vascular disease. We have found it most commonly in patients who have a chronic internal carotid artery occlusion or a severe carotid stenosis. This is consistent with the experience of Hass and Flam.[31] It is probably the result of a slow failure of collateral circulation, either from progressive atherosclerosis or from thombosis superimposed on stenotic collateral vessels. These patients have presented with a history that suggests the presence of a mass. The focal deficit often is most severe in the foot, the watershed zone of perfusion. Our experience with this group is not yet sufficient to formulate a definite judgment regarding the possible role of bypass surgery. The results have been modestly encouraging.

Primary Orthostatic Cerebral Ischemia.—The patient with orthostatic cerebral ischemia must be distinguished from the patient with vertebral-basilar insufficiency and systemic orthostatic hypotension and the elderly patient with dementia. Angiography in patients who had this syndrome, in the series reported by Houser and associates,[23] uniformly demonstrated the presence of bilateral severe carotid artery stenosis or occlusion. It should be emphasized that this group of patients is distinct and separate from

Table 3.—**Role of Bypass Surgery**

Indication	Vessel pathology*	Clinical symptoms
Firm	ICA occlusion or ICA siphon stenosis	TIAs Amaurosis fugax Slow stroke Progressing stroke Orthostatic cerebral ischemia
	Giant ICA aneurysm	Prelude to ICA ligation
	MCA stenosis	TIAs, progressing stroke
Probable	Fibromuscular disease	TIAs, amaurosis fugax
	Moya-Moya	TIAs, slow stroke Small infarctions
Unlikely	ICA occlusion	Prelude to endarterectomy, contralateral side
	ICA occlusion	Major infarction
	ICA occlusion	Asymptomatic
	Diffuse atherosclerosis of intracerebral vasculature	TIAs, infarction

*ICA = internal carotid artery; MCA = middle cerebral artery.

those suffering from senile dementia, which is unlikely to be of vascular origin.

Our experience with bypass surgery for this syndrome has been good. This differs from the work of others, who have reported disappointing results in patients with this constellation of symptoms.[6]

CONCLUSION

It seems to be possible to maintain a patent bypass in the vast majority of properly selected cases[2,4-6,32] and to increase regional cerebral blood flow from the procedure.[33] Perhaps the more difficult task is properly selecting patients and identifying those who would benefit from the operative procedure. Our opinions in this regard largely support the initial conservative estimates about the role of the operation voiced by its founders, Yaşargil and Donaghy. As previously indicated, the procedure should be regarded as primarily a prophylactic operation and not one that will return infarcted brains to life.[2] Although our indications for this operation are still evolving, it is possible to formulate a working table that, in general, follows the indications predicted for the procedure in 1974[34] (Table 3).

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The Use of Procarbazine Hydrochloride Versus Cyclophosphamide in Donor Pretreatment in Cadaveric Renal Transplantation

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During a prospective study, 13 patients received renal transplants from cadaveric donors whose hearts beat up to the time of their death. These donors were pretreated with cyclophosphamide-methylprednisolone (group A) or methylprednisolone-procarbazine hydrochloride (group B). After a minimum follow-up of 12 months, all grafts in group A but only one graft in group B survived. On the basis of this small experience, the combination of cyclophosphamide and methylprednisolone seems to be superior to that of methylprednisolone and procarbazine hydrochloride in reducing allograft immunogenicity. In order to achieve longer survival of the graft in the recipient, pretreatment with procarbazine hydrochloride has been discontinued. Pretreatment of the potential cadaveric allograft donor with cyclophosphamide and methylprednisolone is being continued at our institution.

Because of the shortage of available cadaveric renal allografts, the number of cadaveric renal transplants has not increased during the last 3 years. In addition, survival of renal allografts has not improved during recent years.[1] It seems obvious that optimal use of available renal allografts is necessary. Therefore, quite recently, attempts have been made in animal[2-11] and human[12] models to modify the antigenicity of the allograft in order to improve graft survival. Drugs that have been reported as being effective in reducing allograft immunogenicity are methylprednisolone, cyclophosphamide, and procarbazine hydrochloride. In this present study, we have sought to compare the effectiveness of procarbazine hydrochloride versus cyclophosphamide in pretreatment of the cadaveric human allograft donor, in terms of survival of the allograft.

PATIENTS AND METHODS

Potential cadaveric donors whose hearts were beating without mechanical assistance were pretreated on an alternating basis: group A (regimen A) received 4 g of cyclophosphamide and 4 g of methylprednisolone intravenously 5½ and 2½ hours before the kidney was removed, and group B (regimen B) received 4 g of procarbazine hydrochloride and 4 g of methylprednisolone intravenously 5½ and 2½ hours before the kidney was removed. A total of eight patients received kidneys that were pretreated with regimen A, and five patients received kidneys from donors who were pretreated with regimen B. Both groups had similar matching results and preservation times (Table 1). However, there was a higher percentage of patients at increased risk (juvenile diabetics, patients with more than 50% preformed antibodies, and patients 45 years old or older) in group A than in group B. Patients in both groups received the same standard post-transplant immunosuppressive protocol of intravenous methylprednisolone and intravenous cyclophosphamide, with or without azathioprine and local graft irradiation for rejections.

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Table 1.—**Comparison of Graft and Patient Survival Between Two Groups of Patients Undergoing Kidney Transplantation**

Case	Age (yr); sex	Preformed antibodies (%)	Match	Preservation (hours)	Risk	Survival		Follow-up (months)
						Graft	Patient	
				Group A*				
1	F, 31	88	E_1	3	High	Yes	Yes	19
2	M, 46	0	E	12	High	Yes	Yes	19
3	M, 46	48	E	6	High	Yes	Yes	18
4	F, 15	0	D	9	Normal	Yes	Yes	18
5	M, 27	0	E	6	Normal	Yes	Yes	14
6	M, 35	0	E	2½	Normal	Yes	Yes	14
7†	F, 29	0	E_1	3½	High	Yes	Yes	12
8†	M, 47	1	E	1	High	Yes	Yes	12
				Group B‡				
9	M, 24	2	E_1	3½	Normal	No	No	<3
10	F, 27	0	D	4	Normal	No	Yes	. . .
11†	M, 30	1	E_1	4	High	Yes	Yes	12
12	F, 42	0	E_1	6	Normal	No	Yes	. . .
13	M, 28	0	E	3½	Normal	No	Yes	. . .

*Group A received cyclophosphamide-methylprednisolone pretreated cadaveric kidney.
†Patient had juvenile diabetes mellitus.
‡Group B received procarbazine hydrochloride-methylprednisolone pretreated cadaveric kidney.

RESULTS

All patients and grafts in group A have been at risk for more than 12 months and are surviving with good renal function at the time of this review. Of the five patients who received kidneys pretreated with procarbazine hydrochloride and methylprednisolone, only one has a functioning graft. One patient died 84 days after transplantation. Death was due to anoxic brain injury sustained at the time of marsupialization of a lymphocele. The patient had had a severe delayed hyperacute rejection that ruptured the kidney but, at the time of death, the kidney was functioning. The remaining three patients are surviving; however, all have lost their grafts.

Fig. 1. Serum creatinine levels and graft survival over 90-day period after transplantation in eight patients receiving cyclophosphamide-methylprednisolone (CY/MP) pretreated kidneys (group A) and in five patients receiving procarbazine-methylprednisolone (Proc/MP) pretreated kidneys (group B).

Five patients of group A did not experience any rejection episodes (Fig. 1) and the remaining three have had only mild episodes that were easily controlled. Two patients in group A needed early hemodialysis because of cold damage to the grafts sustained at the time of storage in slush. These results are in contrast to those in group B, in which all patients but one experienced severe rejection episodes necessitating hemodialysis (Fig. 1), with the loss of three kidneys in a delayed hyperacute fashion in the early postoperative period, between the 7th and 12th postoperative day.

DISCUSSION

Early animal experience by Steinmuller[13] and by Elkins and Guttmann[3] showed the importance of the so-called passenger leukocytes within the allograft. Experimental designs of bone marrow chimeras in the rat seemed to point to donor cells of hemopoietic origin as the trigger site of immunogenicity. That grafts deficient in donor leukocytes were also deficient in immunizing capacity was observed by Steinmuller and Hart.[8] These leukocytes seemed to be sensitive to drugs[6,8] and radiation[9] treatment, and prolonged graft survival has been observed by Steinmuller and Hart,[8] Guttmann and Lindquist,[6] and Zincke and Woods[11] after pretreating the donor with cytotoxic agents. The cytotoxic agents used in most

experiments have been cyclophosphamide and procarbazine hydrochloride.

The usefulness of cyclophosphamide in renal transplantation in animals has been demonstrated by Kawabe and his colleagues[14] and by others[6,8] and in humans by Guttmann and his associates.[12] Procarbazine hydrochloride has been used successfully in pretreatment in renal transplantation by Guttmann and Lindquist[6] and by us[11] in animals. Shehadeh and associates[15] could demonstrate that cyclophosphamide was the most effective in modifying the pathologic features of allograft rejection as compared with methylprednisolone and procarbazine hydrochloride. Furthermore, Guttmann[16] showed that pretreatment with cyclophosphamide caused decreased serologically detectable transplant antigens. The action of procarbazine hydrochloride is probably similar to that of cyclophosphamide. A time-dependent effect of these drugs on lymphocyte response to phytohemagglutinin has been demonstrated.[12,17] Procarbazine hydrochloride, a methylhydrazine derivative, seems to act predominantly on the thymus-derived cell population.[18] The beneficial effect of methylprednisolone on ischemic kidneys[19] may contribute to a better early allograft function although suppression of lymphocyte blastogenesis also has been observed when larger doses were used.[20] We have suggested previously[11] that these cytotoxic agents and methylprednisolone may cause a modification of the antigenic properties of passenger leukocytes and parenchymal cells per se. On the other hand, Zincke and associates[21] showed that cyclophosphamide, procarbazine hydrochloride, and methylprednisolone, as used in the dosages described above, did not cause any structural changes on human and canine renal allografts as demonstrated on histopathologic examination. Guttmann and associates[12] showed a 2-year graft survival of nearly 80% when the human cadaveric donor was pretreated with high doses of cyclophosphamide and methylprednisolone before removal of the kidney. In our laboratory,[11] pretreatment of donor dogs with procarbazine hydrochloride was more successful in prolonging graft and recipient survival than was methylprednisolone or cyclophosphamide.

Thus, on the basis of our laboratory findings this present study was performed. Our results appear to demonstrate that, at least in these small groups, patients who received cadaveric kidneys pretreated with cyclophosphamide and methylprednisolone in high doses before the kidneys were removed had rejection episodes that were less severe and fewer in number than did patients who received kidneys pretreated with procarbazine hydrochloride and methylprednisolone. The pretreatment with cyclophosphamide and methylprednisolone provided excellent graft survival with good function. These findings support the recent report by Guttmann and associates.[12] Whereas procarbazine hydrochloride, alone and in combination with methylprednisolone, has proved to be the most successful drug in the pretreatment of the donor dog,[11] in our experience this agent was not effective in decreasing the number and severity of rejection episodes in the human recipient. The discrepancy between the animal and human results might be explainable as a species difference. Pretreatment of the potential cadaveric human allograft donor who has a beating heart with cyclophosphamide and methylprednisolone is being continued at our institution. Pretreatment with procarbazine hydrochloride has been discontinued.

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Endomyocardiopathy With Eosinophilia

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Five patients were seen at the Mayo Clinic over an 8-year period with the following complex of clinical and morphologic features: striking eosinophilia, cardiomyopathy, hepatosplenomegaly, and either a rapidly fatal or a prolonged, debilitating illness. In recent years, controversy has raged over the precise designation of this syndrome, with proposals ranging from eosinophilic leukemia to hypereosinophilic syndromes. To focus on the major target organ of the disease, we have favored the term endomyocardiopathy with eosinophilia. Experience with these five patients showed that (1) eosinophilia can persist for many years before symptoms appear; (2) progressive restrictive cardiac disease was the major cause of death and debility; (3) osmiophilic cytoplasmic inclusions are present in eosinophils of these patients and also in cells from other patients with marked eosinophilia; and (4) echocardiography may prove to be a useful noninvasive tool to diagnose and follow the progress of cardiac involvement. Although none of these patients was thought to have leukemia, intensive therapy with steroids or cytotoxic agents, or both, is considered necessary to control the progression of the disease.

Despite recognition of the eosinophil as a distinctive cell for over 100 years,[1] its specific functions have not been defined, although a recent report[2] implies an antiparasitic role. To compound the mystery, significant eosinophilia can be found in such diverse conditions as bronchial asthma, parasitic infestations, polyarteritis nodosa, rheumatoid arthritis, Hodgkin's disease, and various dermatitides. In addition, marked eosinophilia can be associated with various diseases in which the major tissue eosinophilic involvement is focused on a specific target organ; for example, eosinophilic gastroenteritis,[3] Löffler's syndrome of the lung,[4] eosinophilic cystitis,[5] and hepatic involvement with eosinophilia.[6] There have also been many reports in the literature concerning a distinctive entity that is characterized by striking eosinophilia, infiltration of various organs (especially the heart) by "mature" eosinophils with associated necrosis or fibrosis, or both, and a progressively unfavorable clinical course. Some authors believe that such a syndrome justifies a diagnosis of eosinophilic leukemia[7] whereas others have preferred more benign designations such as disseminated eosinophilic collagen disease,[8] Löffler's endocarditis parietalis fibroplastica,[9] endomyocardial fibrosis with eosinophilia,[10] or the all-embracing term "hypereosinophilic syndromes."[11]

This report concerns five patients seen at the Mayo Clinic between 1967 and 1974 who showed the following complex of clinical and morphologic findings: striking eosinophilia, cardiomyopathy, hepatosplenomegaly, and either a rapidly fatal or a prolonged debilitating illness. In reporting these cases, we would like to stress the features of the syndrome, illustrate the procedures that make the diagnosis possible, and detail our experience with therapy.

REPORT OF CASES

Brief case histories of the five patients follow. Specific laboratory studies are detailed in Tables 1 through 4.

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Case 1.—A 17-year-old male student developed chest pains and malaise late in 1966. These symptoms increased in September 1967 and at that time, despite the absence of specific physical abnormality, he was found to be anemic and to have marked eosinophilia. He was transfused with two units of blood and began to take a cortisone preparation. One month later, arterial occlusion suddenly involved both of his lower extremities. At surgery, complete aortic obstruction was found, caused by a saddle embolus at the aorto-iliac junction. In addition, splenomegaly was noted. Following embolectomy, the arterial supply improved only temporarily; therefore, 2 days later he was transferred to the Mayo Clinic for further treatment. He had sinus tachycardia, grade 2/6 ejection systolic murmur at the left sternal border, and impaired arterial pulsations in his legs. Subsequent removal of fibrinous material via bilateral common femoral arteriotomies resulted in restoration of the circulation. Because of persistence of debility and marked eosinophilia, a diagnosis of polyarteritis nodosa was considered. Thus, treatment with prednisone, 120 mg/day, was begun and was reduced to 60 mg/day 2 weeks later; he also received oral anticoagulant therapy. During the first few months of 1968 his progress was good and, although the eosinophilia persisted (for example, 32% of a total leukocyte count of 9,200 in January), the dosage of prednisone was reduced gradually and the drug was discontinued in April after 7 months of therapy.

In August 1968, he presented with a 4-week history of nausea, vomiting, diarrhea, and weight loss. No cardiac murmurs were detected then but his spleen was just palpable. Prednisone was prescribed (10 mg/day) as well as diphenhydramine, 100 mg four times daily. The latter drug relieved his nausea and there was a decrease in eosinophilia and moderate improvement in his cough and dyspnea. These symptoms recurred when diphenhydramine was discontinued. He was admitted for the last time in November 1968 with marked anorexia, weight loss, and persistent coughing. On examination, the venous pressure was moderately elevated. The precordium was hyperactive. The pulmonic component of the second heart sound was moderately accentuated. He had tachycardia with summation gallop, a grade 3/6 pansystolic apical murmur, and, in addition, a grade 2/6 pansystolic murmur at the left sternal border radiating to the right sternal border which increased with inspiration. The liver was palpated 2 cm below the right costal margin and was nonpulsatile. *Staphylococcus aureus* was cultured from his blood. Despite intensive therapy, he showed progressive deterioration until his death 1 month later.

At autopsy, the most striking finding was the presence of a massive mural thrombus that virtually filled the left ventricular cavity of the heart. The endocardium of the right ventricle was markedly thickened. Focal scarring was seen in the myocardium of both ventricles with foci of acute inflammation on the left. Both kidneys had many small infarcts and the glomeruli showed marked recent proliferative changes. Eosinophilic hyperplasia was present in the bone marrow. The spleen was greatly enlarged, weighing 830 g, but apart from a recent infarct and marked infiltration by mature eosinophils, its architecture was preserved.

Case 2.—A 52-year-old male paper mill worker developed a chronic cough and easy fatigability in 1964. Absolute eosinophilia was noted at this time. Two years later he had increasing fatigue and night sweats in addition to the persistent cough. He was found to have an ejection systolic murmur at the base of the heart but no other physical abnormality. Studies by his local physicians revealed a marked peripheral eosinophilia and eosinophilic hyperplasia in the bone marrow. The leukocyte alkaline phosphatase (LAP) scores were low—30 and 45 (compared to a control of 103).

In October 1968, he was admitted with shortness of breath, fatigue, and diarrhea. There were no abnormal physical findings but he was found to have eosinophilia and significant proteinuria plus reduced creatinine clearance. Renal biopsy specimen showed membranous glomerular changes with marked interstitial fibrosis and tubular atrophy. He was, therefore, given a trial of prednisone, 20 mg/day.

By November 1968, he noted increasing breathlessness and ankle edema. There was marked elevation of the jugular venous pressure, with the dominant venous wave being a rapid Y descent. A positive Kussmaul's sign was noted. The second heart sound was normal and there was no accentuation of the pulmonic component. Both atrial and ventricular gallops were noted. There was a late crescendo grade 1/6 apical systolic murmur. There were no abnormalities found on auscultating the lungs. The liver was palpated 8 cm below the right costal margin and the tip of the spleen was palpable. Anticoagulants and azathioprine (50 mg/day) were begun in addition to digoxin and prednisone. The steroid was gradually tapered over the next 3 months.

He was admitted for his terminal illness in February 1969 with increasing breathlessness and a dry hacking cough. Severe cardiac failure was evident and despite intensive therapy, progressive renal failure ensued and he died 2 weeks later.

At autopsy, the heart weighed 500 g. Focal fibrosis of the myocardium, endocardial fibrosis of both ventricles, and extensive mural thrombi involving both ventricles and the right atrial appendage were noted. Multiple septic emboli were found in many organs, *Staphylococcus aureus* being cultured from these lesions as well as from the blood in the heart. The spleen weighed 405 g; microscopically, the pulp was hemorrhagic. The liver was passively congested; the kidneys showed many septic emboli as well as nephrocalcinosis.

Case 3.—A 16-year-old female student developed fatigue and abdominal fullness in December 1970. Physical examination elsewhere 2 months later revealed a grade 4/6 systolic murmur (previously undetected), cardiomegaly, and an enlarged liver. Laboratory studies at the time showed anemia (hemoglobin, 9 g/dl) and a striking eosinophilia with mostly mature cells, but with a few myelocytes and metamyelocytes. Eosinophilic hyperplasia was noted in the bone marrow. The leukocyte alkaline phosphatase score was normal and a direct marrow preparation failed to reveal the Philadelphia (Ph^1) chromosome. Busulfan therapy was begun in March and continued for the next 3½ months. In November 1971, ascites was detected and the presence of a pericardial effusion was suspected from the globular cardiac configuration on the roentgenogram of the chest. Marked eosinophilia was still present although the bone marrow showed a lesser degree of eosinophilic and myeloid hyperplasia than in the earlier study. On

this occasion, a suspected Ph[1] chromosome was detected in a few cells on a direct marrow preparation.

At the time of her first visit to the Mayo Clinic in December 1971, her presenting symptoms were those of lethargy and exertional dyspnea. The jugular venous pressure was moderately elevated with the prominent wave being a V wave with rapid Y descent. The precordium was overactive. There was moderate accentuation of the pulmonic component. A prominent ventricular gallop was present in addition to a grade 4/6 pansystolic apical murmur that radiated into the left axilla. The lungs were clear. The liver was palpable and ascites was present. A diagnosis of restrictive cardiomyopathy with incompetence of the mitral valve was made. On dismissal from the clinic, her therapy consisted of digoxin, furosemide, potassium supplements, and a sodium-restricted diet.

She has been followed up by her physician at 3- to 6-month intervals. Further chromosomal studies of direct bone marrow preparations have been reported to be normal. She continued to have absolute eosinophilia for at least 9 months, but between October 1972 and our last reported value in June 1974, her eosinophil count has been normal. Atrial fibrillation developed in April 1973 and has persisted. Her local physician reported in September 1974 that her exercise tolerance was adequate, although there was evidence of gross mitral and tricuspid regurgitation. She was being treated with digoxin, furosemide, spironolactone, and a thiazide.

Case 4.—A 50-year-old male engineer had been known to have an elevated eosinophil count since 1969—about 40% of a total leukocyte count of 15,000. Apart from having mild diabetes mellitus and, in the past, a number of episodes of renal colic, he was in good health. In 1973 thrombocytopenia developed. Since this proved unresponsive to steroid therapy, splenectomy was performed in July of that year. The spleen weighed 1,850 g and was infiltrated massively by eosinophils, most of which were mature, but a few immature forms including metamyelocytes were seen. His postoperative course was complicated by the development of a subdiaphragmatic hematoma.

In September 1973, he developed diffuse body aching and increasing malaise. Physical examination 1 month later revealed that the jugular venous pressure was slightly elevated, the predominant wave being an A wave. The first and second heart sounds were normal. He had a grade 2/6 pansystolic harsh apical murmur transmitted to the left axilla. The murmur decreased when he inhaled amyl nitrite. No diastolic murmur was heard. Lung fields were clear at auscultation. The liver was thought to be just palpable below the right costal margin. His elbows, ankles, and knees were moderately swollen and tender and his calf and forearm muscles were painful. The significant laboratory abnormalities were those of marked peripheral eosinophilia, bone marrow eosinophilic hyperplasia, and elevations of serum vitamin B_{12} and of IgG. An echocardiogram demonstrated moderate to marked thickening of the walls of both ventricles, a pattern suggestive of infiltrative cardiomyopathy or mural thrombosis, or both.

While undergoing evaluation, his dyspnea worsened and peripheral edema appeared. His jugular venous pressure was elevated, a cardiac gallop rhythm was present, and the liver was larger, extending to 6 cm distal to the right costal margin. Treatment with digoxin and furosemide gave rapid relief. Also, during this evaluation his leukocyte count decreased from 124,000 to 21,000, without treatment, over a 2-week period, while the differential cell counts remained essentially in the same proportions. However, his hemoglobin also decreased from 14.9 to 10.6 g/dl. A review of the splenic tissue removed in July suggested the presence of a myeloproliferative disorder, possibly a variant of chronic granulocytic leukemia. For this reason, a trial of hydroxyurea was begun late in November 1973. A week later, however, ascites, ankle edema, and severe pruritus became evident. Over the next 4 months his condition gradually deteriorated. Thrombocytopenia became evident and worsened despite discontinuation of the hydroxyurea. From January until his death in March 1974, his course was one of intractable cardiac failure.

At autopsy, the heart was markedly enlarged—780 g in weight. The most striking abnormality was a nodular deposition of fibrin and thrombus involving the entire endocardial surface of both ventricles and occupying about 50% of the capacity of each ventricle. There was some extension of thrombus onto the base of the pulmonary valve. A large hemorrhagic vegetation was found on the anterior cusp of the mitral valve. The liver weighed 3,400 g. A right pleural effusion was present, whereas the left lung was covered by thick fibrous pleura. On microscopic examination, fibrin-platelet material covered the endocardial surface; deep to this layer there was a proliferation of connective tissue not only in the endocardium but also extending into the myocardium, which showed a marked infiltrate of eosinophils and round cells. Sections of the liver showed areas of extramedullary erythropoiesis and ventricular necrosis secondary to congestive cardiac failure. Eosinophilic infiltrates were prominent in lymph nodes, liver, kidneys, skin, pancreas, and lung.

Case 5.—A 26-year-old male steelworker presented at the Mayo Clinic in May 1974 with the chief complaint of progressive fatigability of 4 months' duration and increasing exertional dyspnea for 3 months. Investigation elsewhere had revealed the presence of a cardiac murmur, not previously detected, as well as hepatomegaly, pulmonary congestion, peripheral edema, and marked eosinophilia. Some improvement occurred during therapy with digoxin and diuretics. On physical examination, third and fourth heart sounds were present, as were a grade 3/6 blowing, holosystolic apical murmur, a grade 1/6 short diastolic murmur at the left sternal border, and a pericardial friction rub at the anterior axillary line. His liver measured 13 cm overall in the mid-clavicular line; the spleen was just palpable. The laboratory studies revealed anemia, striking eosinophilia, a low LAP score, elevated vitamin B_{12} and unsaturated B_{12} binding capacity, and increased immature granulocytic elements, especially in the eosinophil line, on bone marrow study (Tables 1 through 3). Treatment with hydroxyurea was begun. Initially, he received two doses of 6 g each, 3 days apart, followed by daily doses of 1 g. He did show a definite response to this treatment—his liver and spleen became impalpable and his leukocyte count fell to 5,400/mm^3 (38% eosinophils). Therapy with digoxin and a diuretic was continued.

Early in June 1974, however, severe diarrhea occurred and persisted for 3 weeks despite cessation of all his medications except furosemide. When reassessed the next month, he thought that he had improved—the diarrhea

was virtually gone and his exercise tolerance was much better. Proximal muscle wasting and pruritus were new developments, however. The venous pressure was markedly elevated, the prominent waves being the X and Y descents, which were very rapid. Cardiac murmurs typical of mitral and tricuspid regurgitation and third and fourth heart sounds were noted. No hepatosplenomegaly was detected. A small bowel roentgenogram was normal. Duodenal biopsy showed increased numbers of eosinophils but not a diffuse infiltrative process. A trial of alternate-day steroids (prednisone, 80 mg in four divided doses every other day) was instituted, in addition to resumption of digoxin and diuretic therapy.

During the next 2 months, his progress was satisfactory. His leukocyte count was maintained in the 14,000 range, and his prednisone dosage was reduced to 20 mg every other day. However, late in September it had increased to 33,000 with 53% eosinophils. Prednisone dosage was increased to 50 mg/day and maintained at that amount until January 1975. Evaluation then revealed that he was able to perform light work but he tended to retain excessive fluid readily. Because there had been no real response to steroid therapy, hydroxyurea (500 mg four times daily) was added. Within 1 week, his leukocyte count decreased from 12,600 to 3,700 and the eosinophil differential decreased from 39% to 7%. Hydroxyurea dosage was tapered over a 3-week period but prednisone was continued, as was digoxin, a diuretic, and diphenhydramine, 100 mg at bedtime (for pruritus). When evaluated last in August 1975, his general health was satisfactory and he had continued working. Clinically, his cardiac status was unchanged but his lung fields were clear and neither the liver nor the spleen was palpable. An echocardiogram was unchanged from the recording made in January. His leukocyte count was 8,700 with 19% eosinophils. It was decided to continue the same therapeutic regimen.

Comments on the Cases.—These five cases show many features common to all. There was a male predominance with a mean age of 32 years. Three patients have died, all within 4 to 5 years of the first symptoms. In the other two, the duration of the illness has been 20 months and 4 years, respectively. In the majority, significant eosinophilia preceded definite cardiac impairment. Easy fatigability and dyspnea were prominent symptoms in each patient, although one (case 1) presented with acute arterial insufficiency of his lower limbs. The heart was the major target organ in all five; each had sinus tachycardia, cardiomegaly, congestive cardiac failure, and systolic murmurs indicative of insufficiency of one or both atrioventricular valves. Endocardial thickening or mural thrombi, or both, were demonstrated in three patients. Hepatosplenomegaly, transient or persistent, was present in all.

Laboratory Studies. Hematologic.—1. Light microscopy: In each case the peripheral blood showed striking eosinophilia (Table 1). Only in case 3 has there been regression of the eosinophilia; indeed, that patient has shown normal leukocyte counts and differential without therapy for at least 2 years.

Under light microscopy, the eosinophils appeared normal in each case. However, close scrutiny revealed that the eosinophils in two of the cases had decreased numbers of granules as well as vacuolation of the cytoplasm. Case 5 showed some hypersegmentation of the eosinophilic nuclei. With the exception of case 4, in which there were significant increases in the basophil counts, there were no other abnormalities in leukocytic morphology. All patients had significant anemia and three developed thrombocytopenia.

Bone marrow differential counts showed increased granulopoiesis, notably in the eosinophilic line. However, cell maturation was orderly, with no patient having a significant increase in blast cells, although case 1 did have a slight excess of immature forms.

2. Electron microscopy: Buffy coats from peripheral blood were fixed and processed for electron microscopy from cases 3, 4, and 5. In addition, bone

Table 1.—**Peripheral Hematologic Data**

Case	Hemoglobin (g/dl)	Total leukocyte count ($\times 10^3$)	Eosinophil differential (%)	Platelet count	Comments on peripheral smear
1	8.5 to 14.1	6.7 to 13.4	15 to 84	Reduced	Majority of eosinophils mature, but some showed reduced numbers of, and irregular, granules
2	10.6 to 14.3	13 to 22.4	45 to 70	Reduced terminally	Eosinophils mature; late in course, thrombocytopenia and fragmented red cells
3	9.5 to 14.5	6.7 to 18.0	20 to 25 (initially)	Normal	Eosinophils mature; moderate red cell macrocytosis
4	9.7 to 15.0	17 to 124.0	9.7 to 15	Reduced (temporary improvement following splenectomy)	Eosinophils appeared mature except for reduced numbers of, and irregular, granules; vacuolation of cytoplasm, mild neutrophilic immaturity
5	7 to 12.0	3.7 to 33.0	7 to 80	Normal	Eosinophils appeared mature, though some had hypersegmentation of the nucleus
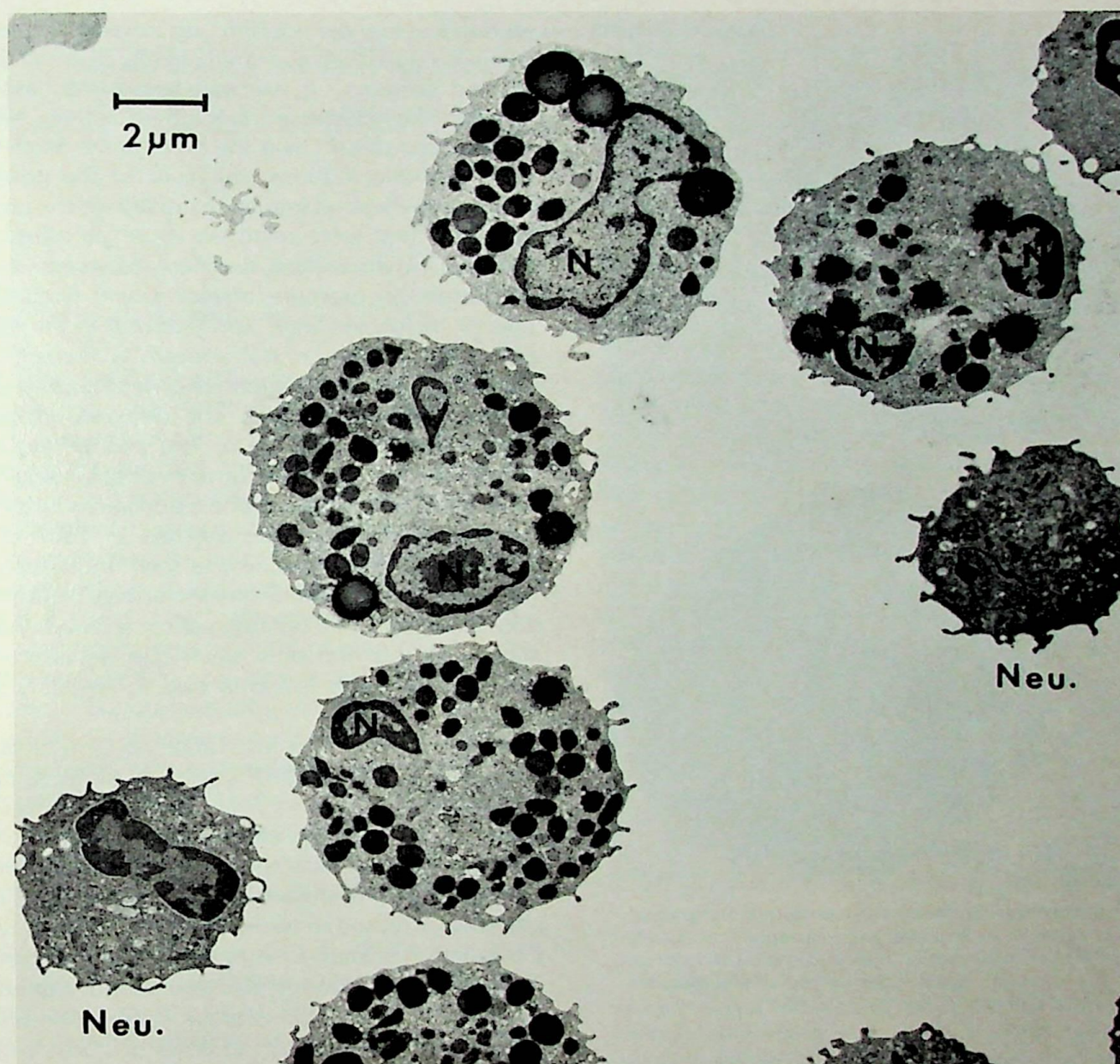
Fig. 1. Survery electron micrograph showing four eosinophils and two neutrophils (*Neu.*) from case 4. In all eosinophils, large and very electron-dense, round, cytoplasmic inclusions or granules are seen. "Specific" eosinophil granules contain central cores; *N* = nuclei. (×5,400.)

marrow particles were similarly processed from case 5. Initial fixation was carried out with 3% glutaraldehyde in phosphate buffer (pH 7.3) for 3 hours at room temperature. The specimens were postfixed in osmic acid, dissolved in phosphate buffer, and embedded in Epon 812. The sections were obtained in an LKB Ultratome III Microtome (LKB Instruments, Rockville, Maryland) and viewed unstained or double-stained with lead citrate and uranyl acetate. In some instances, specimens were reacted for peroxidase after glutaraldehyde fixation, following the procedure of Graham and Karnovsky.[12] These specimens were processed subsequently as described above. A Hitachi HU-12 or Phillips 201 electron microscope was used to examine the specimens.

For the purpose of comparing the morphology of the eosinophils, we studied several patients with marked eosinophilia of different etiologies but without clinical endomyocardiopathy. This group included one patient with reticulum cell sarcoma (no evidence of endomyocardiopathy at autopsy), another with dermatitis and persistently high IgE, and another with a nodular eruption on the legs.

As shown in Figures 1 and 2, the most remarkable feature was the presence of large, round, and homogeneously very electron-dense cytoplasmic inclu-
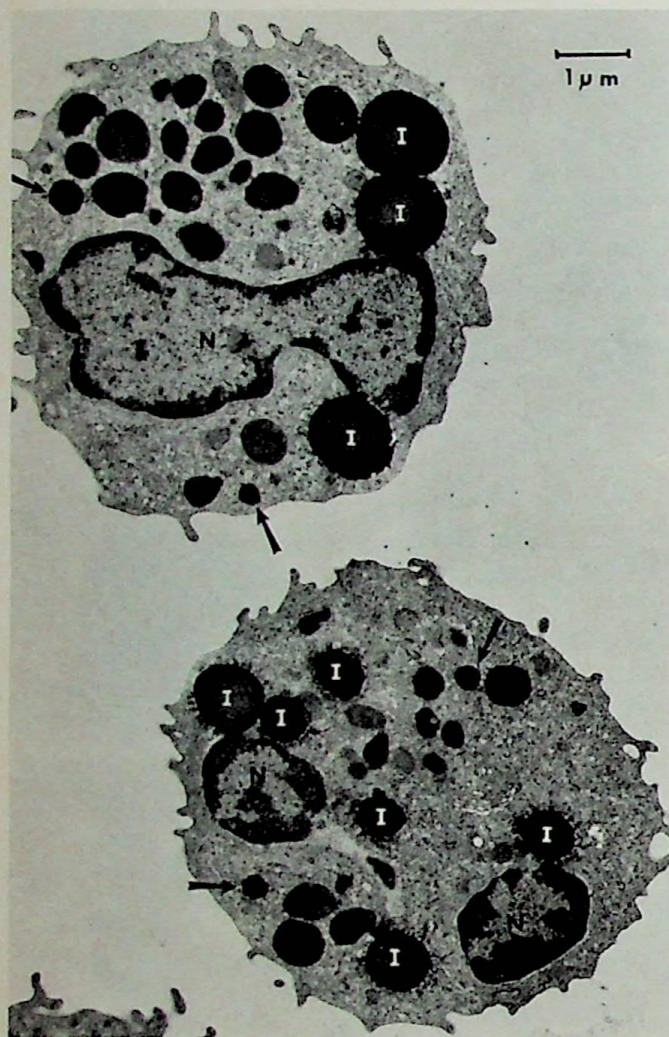
Fig. 2. This electron micrograph (case 4) taken at higher magnification shows in better detail two eosinophils containing large granules or inclusions (*I*) as well as similar granules of smaller size (*arrows*). Again "specific" eosinophil granules possess central cores; *N* = nuclei. (×13,050.)

sions. These inclusions did not possess the characteristic crystalline core of the mature eosinophilic granule. They varied in size but were usually larger than the normal mature granules. The latter often were unevenly distributed and were present in fewer numbers. The homogeneously dense inclusions or granules were osmiophilic or inherently electron dense because they appeared equally dense in the unstained sections. It was not possible to ascertain whether they possessed peroxidase activity because the electron density was the same in the reacted and unreacted preparations. In favor of the possibility that they are indeed immature granules is the observation that they were relatively more prominent and numerous in the earliest, undifferentiated eosinophilic precursors (for example, myelocytes).[13] Similar granules or inclusions were also detected in the eosinophils of the three control patients with marked eosinophilia but without apparent endomyocardiopathy.

3. Cytogenetic studies: The direct bone marrow chromosome preparation of Tjio and Whang[14] was used. Twenty-five consecutive evaluable metaphases were examined, photographed, and karyotyped when possible. The results are detailed in Table 2. No Philadelphia chromosome was seen in these studies, nor were extra chromosomes observed. As can be noted in Table 2, all but one patient showed chromosomal loss but the only significant departure from normal values was found in case 1, probably in the C group.

4. Biochemical studies (Table 3): One of the patients had a reduced LAP score; in addition, case 2 was reported to have a low LAP score before referral. Case 1 had an elevated value. The serum vitamin B_{12} level was increased in four of five patients and the unsaturated vitamin B_{12} binding capacity was increased in two of the three patients tested. Coagulation studies were performed in four patients; although some abnormalities were found, a significant degree of disseminated vascular coagulation was not thought to be present at the times of study, with the possible exception of case 2. That patient has schistocytes, thrombocytopenia, and increased acceleration of the thromboplastin generation test late in his illness.

Cardiac Data (Table 4).—All patients developed clinical evidence of mitral insufficiency, tricuspid

Table 2.—**Direct Bone Marrow Chromosome Studies***

Case	Date	Number of metaphases studied	Chromosome loss by chromosome group						
			A	B	C+X	D	E	F	G+Y†
1	8/15/68	25	0	0	10	2	4	2	0
2	12/19/68	25	0	0	0	0	0	0	2
3	12/2/71	25	0	0	2	1	0	2	4
4	11/1/73	6	0	0	0	0	0	0	0
5	5/9/74	30	0	0	2	0	1	0	0

*Extra chromosomes were not observed in any metaphases of this study.
†No Philadelphia chromosome was seen in any of these five patients.

Table 3.—**Miscellaneous Laboratory Studies**

Case	Leukocyte alkaline phosphatase score (normal, 30-70)	Serum vitamin B_{12} (pg/ml) (normal, 170-760)	Unsaturated B_{12} binding capacity (pg/ml) (normal, 870-1,800)	Abnormalities of coagulation
1	124	>2,250	ND*	Factor VIII, grade 2 acceleration of TGT†
2	57	500	ND	Grade 3 acceleration of TGT
3	60, 95	1,164	1,123	Prothrombin time, partial thromboplastin time
4	39	>1,400	3,380	ND
5	14	>1,400	5,580	Prothrombin time

*ND = no data.
†TGT = thromboplastin generation test.

insufficiency, and congestive heart failure. The chest roentgenogram and electrocardiogram showed nonspecific abnormalities. Cardiac catheterization studies were performed on patients 1, 2, and 3. All had significantly increased wedge pressures compatible with mitral regurgitation, with patients 1 and 2 showing pulmonary hypertension. The cardiac index was low-normal in case 1 and significantly depressed in cases 2 and 3. Left ventriculograms in three patients confirmed the presence of significant mitral insufficiency, and in one case mural thrombus was suspected. Echocardiography was helpful in the clinical evaluation of three patients: in case 3 a large pericardial effusion was detected; in case 4, there was moderate to marked thickening of the walls of both ventricles; and serial echocardiograms done on case 5 showed increasing dimensions of the left atrium and left ventricular cavity over a 6-month period between July 1974 and January 1975.

Pathology (Table 5).—In each of the three cases in which autopsies were performed, the most impressive changes were in the heart. Mural thrombi were present within the left ventricular chamber in all three and in the right ventricle in one. The thrombi were friable and consisted of an inner layer of pale pink fibrin overlaid by a deep red deposit of clotted blood. The border between the two layers was usually indistinct. The thrombus had a distinctly lamellar appearance. The deep parts of the thrombi were firmly attached to a markedly thickened, white endocardium (Fig. 3). Microscopically, the endocardium was seen to consist of vascularized collagen that penetrated, in narrow bands, deeply into the adjacent endocardium. Infiltrating the scar tissue were moderate numbers of lymphocytes with occasional plasma cells and eosinophils. In case 1 (Fig. 4), focal collections of eosinophils were present within the interstitium of the myocardium as well as in muscle cells—giving the appearance of an eosinophilic microabscess. All three hearts had significant bi-

Table 4.—**Cardiac Catheterization Data**

	Pressure		
Measurement	Case 1 (mm Hg)	Case 2 (mm Hg)	Case 3 (mm Hg)
Femoral artery	110/60	130/78	124/83
Wedge	30/12	36/18	32/27-22-27
Pulmonary artery	47/20	48/26	30/21
Right ventricle	45/2-8	46/11-19-22	31/8-23
Mid right atrium	8/0	24/14	27/14
Systemic index (liters/min per m²)	2.6	1.9	1.8
Systemic resistance (units/m²)	29.6	5.0	52.0
Pulmonary resistance (units/m²)	11.2	. . .	13.0

Table 5.—**Summary of Autopsy Data**

Organ	Case 1	Case 2	Case 4
Heart			
Enlargement	+	+	+
Weight, g	430	500	780
Mural thrombus	+	+	+
Endocardial thickening	+	+	+
Myocardial fibrosis	+	+	+
Eosinophilic infiltrate	+	−	+
Lungs			
Pleural effusion	−	+	−
Pleural fibrosis	−	+	+
Pulmonary edema	+	+	−
Eosinophilic infiltrate	−	−	+
Liver			
Enlargement	+	+	+
Weight, g	2,940	2,490	2,400
Centrilobular necrosis	+	+	+
Eosinophilic infiltrate	−	−	+
Spleen			
Enlargement	+	+	+
Weight, g	830	405	1,800
Congestion of pulp	+	+	+
Eosinophilic infiltrate	+	−	+
Kidneys			
Glomerulopathy	Focal proliferation	Membranous	Membranous
Eosinophilic infiltrate	−	−	+

Fig. 3. Left ventricular cavity in endomyocardiopathy with eosinophilia (case 2). Cavity is nearly filled with granular friable thrombus firmly attached to thickened endocardium.

ventricular hypertrophy. Valvular abnormalities were present only in case 4, as indicated in the case report.

In two of the patients (cases 1 and 4), the bone marrow was markedly hypercellular, containing increased numbers of mature eosinophils as well as eosinophilic metamyelocytes and earlier forms of the myeloid series. The latter were particularly prominent in case 1. Megakaryocytes were rarely encountered in these two cases and erythropoiesis was significantly decreased. In case 2, the bone marrow was normocellular and had the usual number of megakaryocytes and erythroid elements. Mature eosinophils, however, were somewhat increased in number.

The glomeruli in all three cases studied were abnormal. Two (cases 2 and 4) contained early membranous changes and, in case 1, focal proliferation was present. These alterations were not striking. Although hepatomegaly was present in each of the cases, an eosinophilic infiltrate was present within the portal spaces only in case 4. Splenomegaly was also prominent in all three, with significantly increased numbers of eosinophils within the sinusoids of cases 1 and 4. The media of the small arteries in the lungs of case 1 was significantly thickened, resembling the changes seen in patients with sustained pulmonary hypertension.

Miscellaneous.—A number of other tests were performed on all or some of the group: serum protein electrophoresis, hepatitis-associated antigen, antinuclear antibody, uric acid, *Trichinella* skin test, and stool examinations for ova and parasites. None showed abnormal values. Serum immunoglobulins were measured in cases 4 and 5, the former showing raised IgM (2.95 mg/ml; normal, 0.2 to 1.4), the latter increased IgG (19.06 mg/ml; normal, 6.4 to 14.3). It is of interest that, in the two patients in whom the serum IgE was measured, it was found to be low in each—<3.7 ng/ml in case 4 and 9.5 ng/ml in case 5 (normal, 6 to 780 ng/ml).

Treatment.—Although the illness appears to have reached an inactive stage in one patient, the therapeutic regimens have been generally disappointing. Treatment with immunosuppressive or cytotoxic agents, or both, was used in each patient, as well as the usual methods to control congestive cardiac failure. None of the three who died gained noticeable relief from the use of steroids, azathioprine, or hydroxyurea. Diphenhydramine appeared temporarily beneficial in case 1 but gave no relief in case 2. Of the two survivors to date, case 3 was treated with busulfan but without recordable benefit during the thera-

Fig. 4. Myocardium from case 1; focal collection of cells, mainly eosinophils, is present with muscle cell damage and infiltration into interstitium. (Hematoxylin and eosin; ×205.)

peutic period. Resolution of her eosinophilia came 12 months later, while on no specific therapy. However, severe restrictive cardiopathy with incompetent atrioventricular valves persists. The remaining patient (case 5) does appear to have responded to hydroxyurea on two occasions—eosinophil counts were lowered and his hepatosplenomegaly decreased. For the past 12 months, he has been maintained with prednisone (50 mg daily) with apparent stabilization of his cardiac status. In two of the fatal cases, anticoagulant therapy was used but it did not seem to alter the course of the illness.

DISCUSSION

We believe that each of these five patients has suffered from the same disease entity. In essence, we see a young to middle-aged person, usually a man, who has severe restrictive heart disease, striking and persistent eosinophilia, and a generally unfavorable clinical course despite intensive therapy. A search of the medical literature reveals over 100 case reports of disease entities designated as eosinophilic leukemia,[7] disseminated eosinophilic collagen disease,[8] Löffler's endocarditis parietalis fibroplastica,[9] endomyocardial fibrosis and eosinophilia,[10] and hypereosinophilic syndromes.[11] Included in these are many patients—indeed, a majority—who would fit the clinical picture described above. We should like to describe briefly the characteristics of the above diseases.

In 1969, Benvenisti and Ultmann[15] reviewed 48 autopsy case reports (including 5 of their own) of patients believed to have had eosinophilic leukemia. Selection was based on the following criteria: hepatosplenomegaly, lymphadenopathy, and marked persistent eosinophilia, usually with anemia and thrombocytopenia. Males predominated and most were younger than 50 years of age. Heart failure, respiratory distress, central nervous system dysfunction, and fever were typical manifestations. Therapy failed in all instances, 80% dying within 12 months of diagnosis. At autopsy, the liver, spleen, lymph nodes, heart, lungs, and central nervous system were infiltrated with eosinophils. The presence of immature eosinophils, including increased numbers of blast cells, was observed in the infiltrates in 27 cases. Mural thrombi were noted in 30%, often associated with endomyocardial fibrosis. Obviously, many of these patients had a disease similar to those in our group.

Brink and Weber[16] reviewed 40 autopsy cases of endocarditis parietalis fibroplastica (of Löffler) reported in the literature up until 1962. (Included in the series were 26 cases that had previously been reviewed by Weiss-Carmine.[17]) Of these, men outnumbered women three to one. In 30 patients, death occurred within 2 years of the onset of the illness. All but three had significant eosinophilia, most having eosinophilic infiltration of various organs. The usual clinical picture was that of congestive heart failure, often with systolic murmurs typical of mitral or tricuspid insufficiency, or both. Most had mural thrombi superimposed on thick, fibrous endocardium of either or both ventricles, with extension of inflammation or fibrosis into the adjacent myocardium. Vascular lesions of the small coronary arteries (fibrinoid necrosis, vasculitis, internal fibrous thickening) were noted in over half of the cases.

Endomyocardial fibrosis (EMF) is a form of heart disease widespread throughout equatorial Africa, with an incidence about the same as that of rheumatic heart disease.[18] The cardiac abnormalities are similar to those described in Löffler's endocarditis—that is, marked thickening of the endocardium with scarring of the inner myocardium of one or both ventricles. The papillary muscles are also involved, so that the mitral and tricuspid valves are often rendered incompetent. In a careful review by Ive and associates,[18] 43% of 47 Nigerian patients also had significant eosinophilia and there seemed to be a close relationship between EMF and filariasis. However, in another review by Brockington and associates[19] of 20 Europeans who had EMF and who also had lived in equatorial Africa, 18 had marked elevations of the peripheral blood eosinophil counts. (No comments were made concerning abnormalities in eosinophilic morphology.) In the latter series all the patients were men, and the average age was 34 years.

In 1956, Engfeldt and Zetterström[8] reported the cases of two children with marked eosinophilia and infiltration by eosinophils of various organs, especially the heart, lungs, central nervous system, skin, and skeletal muscles. One of the patients died in acute cardiac failure, the heart showing mural thrombus and thickened endocardium with inflammation and fibrosis of the myocardium. The other was shown clinically to have cardiac involvement. In particular, they stressed the presence of changes in the small blood vessels, not only in the heart but in other tissues—for example, thrombosis and endarteritic narrowing. As both patients also had hypergammaglobulinemia, the authors favored the term "disseminated collagen vascular disease" (DECD) to implicate an autoimmune etiology. Subsequently, other authors[20,21] have favored such a designation for endomyocardiopathy and striking eosinophilia, with addi-

tional involvement of other organs such as the lungs, skin, and skeletal muscle.

Thus, included in the above entities are many patients with similar clinical and pathologic features, albeit with minor variations. Roberts and associates[10] proposed that each reflects the same disease at different stages of development. Hardy and Anderson[11] also believed that each of these disease entities formed part of a continuum and therefore proposed the term "hypereosinophilic syndromes" to encompass the group. Such a broad term has gained some acceptance but we are in accord with Zucker-Franklin[22] in believing that it is too nonspecific. An excellent review of the hypereosinophilic syndrome was recently published by Chusid and associates.[23] Fourteen patients were studied and all were found to have some cardiac abnormality, even though no abnormality was suspected in some members of the group. This study suggests that cardiac involvement may be more prevalent than previously recognized in diseases associated with marked eosinophilia. Thus, there may be some justification for retaining the designation hypereosinophilic syndrome, but we prefer to use the term "endomyocardiopathy with eosinophilia" in this group of patients, in order to focus on the major site of disease, and therefore follow the usage proposed originally by Roberts and associates.[10]

In considering this spectrum of diseases, one of the central issues of controversy has been the classification of eosinophilic leukemia. In his review of the subject, for example, Bousser[24] doubted that any of the 29 such case reports truly represented eosinophilic leukemia and were probably most indicative of a leukemoid response. Odeberg,[20] studying the reports of 16 additional cases, agreed. Although he believed that many of the cases did have a leukemic process, and even immature eosinophils in peripheral blood and bone marrow in some cases, he believed that the marked eosinophilia represented a reactive phenomenon. In this regard, there are two recent papers reporting eosinophilia associated wtih acute lymphoblastic leukemia.[25,26] Two of the three cases proved to be endomyocardial disease but, in all three, the eosinophilia was suppressed whenever remission of the leukemia occurred. Also, we have a case in our files of a 30-year-old man who presented with thrombophlebitis of his left leg and who was also found to have a systolic murmur, scattered pulmonary infiltrates, hepatosplenomegaly, and marked eosinophilia (20,000 to 30,000 mature eosinophils per cubic millimeter; no immature cells). Subsequently, he developed acute leukemia of indeterminate type and died within 2 weeks of this diagnosis. Autopsy revealed subendocardial and myocardial fibrosis with mural thrombosis of the left ventricle. Both liver and spleen showed passive congestion, the latter also with marked infiltration by mature eosinophils. Thus, as with some of the cases discussed by Odeberg,[20] their patients undoubtedly had leukemia but the leukemic cell lines were different from the eosinophils. From another point of view, eosinophilic leukemia has been considered to be a variant of chronic granulocytic leukemia. Such additional findings as coexisting basophilia,[27-29] elevated serum vitamin B_{12} and B_{12} binding capacity,[30] reduced leukocyte alkaline phosphatase score[31] (present in one of our five patients), and the presence of a Ph^1 chromosome[32] have been used to support this diagnosis. To date, the Ph^1 chromosome, which is almost invariably present in chronic granulocytic leukemia, has been identified only in rare instances of eosinophilic leukemia.[23,29,33] On the other hand, cytogenic studies have shown inconsistent abnormalities[23] (as in our cases). In these five cases, the only consistent abnormalities were shown in the vitamin B_{12} and B_{12} binding capacities (elevated in four and two cases, respectively). Such a finding is considered by Fledelius[34] to separate patients with eosinophilic leukemia from those with benign hypereosinophilic states, but we have recently shown that both of these modalities can be elevated in benign diseases and marked eosinophilia.[35]

None of our five patients appeared to be suffering from a leukemic process. Although the eosinophils in the peripheral blood did show some abnormal characteristics (vacuolation of the cytoplasm and scarcity or increased size of the granules), they were essentially mature. We were impressed by the presence of homogeneously dense bodies noted on electron microscopic study but we also observed such granules in various types of eosinophilias used as controls. Originally, the presence of these granules was believed to be diagnostic of the malignant or fatal eosinophilias[36] but, in addition to our observations, Zucker-Franklin and Grusky[13] have described these granules from human peripheral blood cultures and state that they have observed them in eosinophilias other than those associated with endomyocardial disease. Such granules, therefore, are not peculiar to this spectrum of diseases.

In a retrospective study such as this, we cannot hope to shed much light on the dilemma of the diagnosis of eosinophilic leukemia. Studies of the patients with marked eosinophilia and a leukemic process involving other leukocytic lines suggest that the

eosinophilia is reactive.[26,27] Precise stimulating factors could not be determined, but such studies suggest that the marked eosinophilia is involved in an immune defense phenomenon. Although many of the functions of the eosinophil have not been fully resolved, various workers have shown increased eosinophil production to be involved in both humorally[37] and cellularly[38] mediated immune reactions. Perhaps there is an alteration in a structural protein of the endocardium or the myocardium, or both, possibly by a virus or by a mycoplasma,[39] thus establishing a foreign antigenic stimulus. The eosinophils are then attracted to the region and act in a defensive capacity, rather than possessing inherent cardiotoxic properties, as has been suggested.[40] That extreme persistent eosinophilia is not in itself detrimental to the heart is suggested by a patient now being studied by us who has had an eosinophil count of about 10,000/mm³ continuously for 10 years or more but in whom we cannot demonstrate cardiac abnormality. Because we believe that the marked eosinophilic response is representative of the body's defense against an immunologic insult, we would expect that therapy with steroids or cytotoxic agents, or both, would be the logical weapon to control the disease. However, not only has such therapy proved to be disappointing in most cases but also it has had little effect in reducing eosinophil counts. This finding implies that the stimulus to eosinophilia is a powerful one and that the therapeutic regimens should be intensive.

Finally, we wish to stress the importance of studying carefully any patient found to have extreme persistent eosinophilia. Although this hematologic state may reflect some comparatively benign condition such as bronchial asthma, it also may herald a rapidly fatal illness. If such diseases as polyarteritis nodosa and Hodgkin's disease are not evident, attention must be directed toward thorough cardiac evaluation.

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Host Factors in Chronic Obstructive Pulmonary Disease in an Upper Midwest Rural Community

Design, Case Selection, and Clinical Characteristics in a Matched-Pair Study

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A series of 111 index subjects with chronic obstructive pulmonary disease (COPD) who had forced expiratory volume in 1 second (FEV_1) of 70% or less of that predicted were matched on the basis of age, sex, occupation, and smoking history with control subjects who had an FEV_1 of 85% or more of that predicted. Index and control subjects with seasonal or reversible airway disease were excluded. Men outnumbered women by a ratio of 4.5 to 1. Thirty-five percent of the women and 2% of the men were nonsmokers (0 pack-years). There were three PiZ phenotypes in the index group (two nonsmokers) and none in the controls. PiMZ phenotypes in the index group outnumbered those in the controls by 8 to 5. Host factors that might be important in these closely matched pairs were sought by history, physical examination, and a large battery of laboratory tests. A standard respiratory questionnaire revealed the anticipated significantly higher frequency of cough, phlegm, noisy respiration, and all grades of dyspnea in index subjects. Previous lower respiratory tract infections also were more frequent in index subjects than in controls. There were no detectable differences between groups in the frequency of upper airway infections, nasal polyps, sinus surgery, or reported allergy to any substance. If the British Medical Research Council's definition of chronic bronchitis were applied to our study, about two-thirds of our index subjects and almost one-third of our controls would be considered to have chronic bronchitis. Pack-years of smoking were not significantly associated with the amount and duration of cough and expectoration in male or female index subjects or controls. Significant differences between index and control groups on physical examination included the audible forced expiratory flow time over the trachea, the estimated maximal midexpiratory flow, breath sounds, rales, and total excursion of the hemidiaphragms. An endocrine questionnaire and measurement of blood sex hormones did not give any clues as to the propensity of males to develop COPD. Women with airway obstruction similar to that of men had histories of significantly fewer pack-years than did the men, and there was a much larger proportion of women who never smoked. Further studies, specifically on genetic and immunologic characteristics, are under way to identify potential host factors.

Chronic obstructive pulmonary disease (COPD) includes the disease spectrum of obstructive bronchitis and emphysema and predominantly afflicts older men who usually are heavy smokers. Although the environmental influences on COPD have been extensively investigated,[1] less attention has been given to host factors,[2] the well-known exception being the α_1-antitrypsin deficient states.

To determine the potential host factors for COPD, we undertook a matched-pair study in a rural region with low industrial pollution. The subject pairs were matched for age, sex, occupation, and smoking history. This report concerns the design, subject selection, matching, and the clinical characteristics as they pertain to pulmonary function.

SUBJECT SELECTION

Only subjects who lived in a 12-county area in southeastern Minnesota (Fig. 1) were considered. An index subject with COPD was defined as a subject between 45 and 60 years of age who had a forced expiratory volume in 1 second (FEV_1) of 70% or less of that predicted, based on the no-disease formulas of Ferris and associates.[3] Index subjects were selected

This investigation was supported by Contract 1-HR-22942 from the National Heart and Lung Institute. This study was approved by the Mayo Clinic Committee on Human Experimentation, and all participants gave their voluntary consent after having been informed of the details of the investigation.

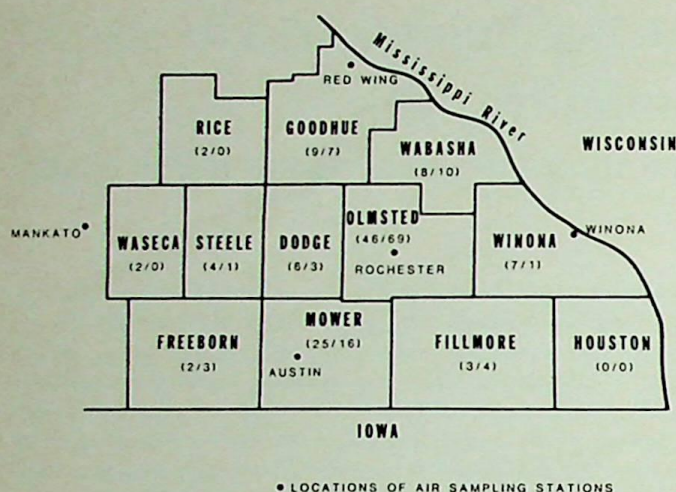



Fig. 1. Source of subjects from the 12-county area in southeastern Minnesota. The numbers in parentheses represent index cases over number of control cases. Solid dots represent locations of air-sampling stations.

on the bases of the low FEV_1 and a physician's diagnosis of chronic as opposed to reversible obstructive pulmonary disease. If a diagnosis of asthma had previously been made (and not bronchitis, emphysema, or COPD) the patient was not included in the study. Once studied, no patient was excluded for reporting "allergy" to one or more substances. An FEV_1 of 85% or more of that predicted was established for subjects who were considered as matched controls. Potential index subjects were chosen from the Mayo Clinic diagnostic files and from the files of physicians practicing in the area (Fig. 2). Nonresponders after follow-up letters were considered refusals. Potential control subjects were selected from a diagnostic file of patients with fractures treated or dental extractions carried out at the Mayo Clinic or with fractures treated by physicians in the area whose patients had been included as index subjects. Matched pairs were from the same record source (Mayo Clinic or non-Mayo Clinic) in 79 instances. The results were similar in those pairs having matched (79) and unmatched (32) record sources. Twenty-one of the 111 index subjects could not be matched for occupation by the method described. To match these 21 subjects, we systematically contacted workers in the occupational categories through the state employment office until we found a control subject of the appropriate age and sex with a comparable smoking history.

A letter of invitation was mailed to each prospective index and control subject. Among those responding to the invitation and having pulmonary function tests, only two potential index subjects and one potential control refused to participate.

By design, index subjects and their matched controls were separated by at least 15% of predicted FEV_1 so as to have two distinct groups: one group with COPD and a normal control group (Fig. 3). We also used the prediction formulas for nonsmokers of Ferris and associates,[3] Kory and associates,[4] Morris and associates,[5] and Berglund and associates[6] and found that index subjects and controls were also separated by these formulas.

METHODS

The FEV_1 was determined by obtaining forced expiratory flow-volume loops using a wedge spirometer (Med Science 270, Med Science, St. Louis, MO) and incorporating a 1-second timer.[7] From the flow-volume loop, the following measurements were determined: the peak expiratory flow (PEF) and the maximal expiratory flow (MEF) at 25% intervals (MEF_{25}, MEF_{50}, MEF_{75}) down the forced vital capacity (FVC) from maximal inspiration. The FEV_1, PEF, MEF_{25}, MEF_{50}, MEF_{75}, and FVC were determined by averaging the last three of five satisfactory efforts. Only in controls was a single-breath nitrogen curve done.[8] The average of three satisfactory efforts was used to determine the "closing volume"[9] and the slope of the nitrogen plateau.[10]

Each subject whose FEV_1 appropriately classified him as index or control subject responded to a verbally administered health questionnaire and provided a family history. Each had a cardiopulmonary physical examination and posteroanterior and lateral thoracic roentgenograms to exclude specific pulmonary disease and to determine total lung capacity (TLC).[11] On the same morning, blood was drawn for a large number of genetic, immunologic, and endocrine studies. Detailed analysis of these studies is to be reported separately. Initial pulmonary function studies were completed by 9:00 a.m. each day. Subjects who had histories of seasonal respiratory difficulty or dyspnea precipitated by exposure to extrinsic allergens suggestive of reversible airway disease were excluded from both the index and the control groups.

MATCHING

Of 111 matched pairs studied between February 1973 and December 1974, 91 were men and 20 were women.

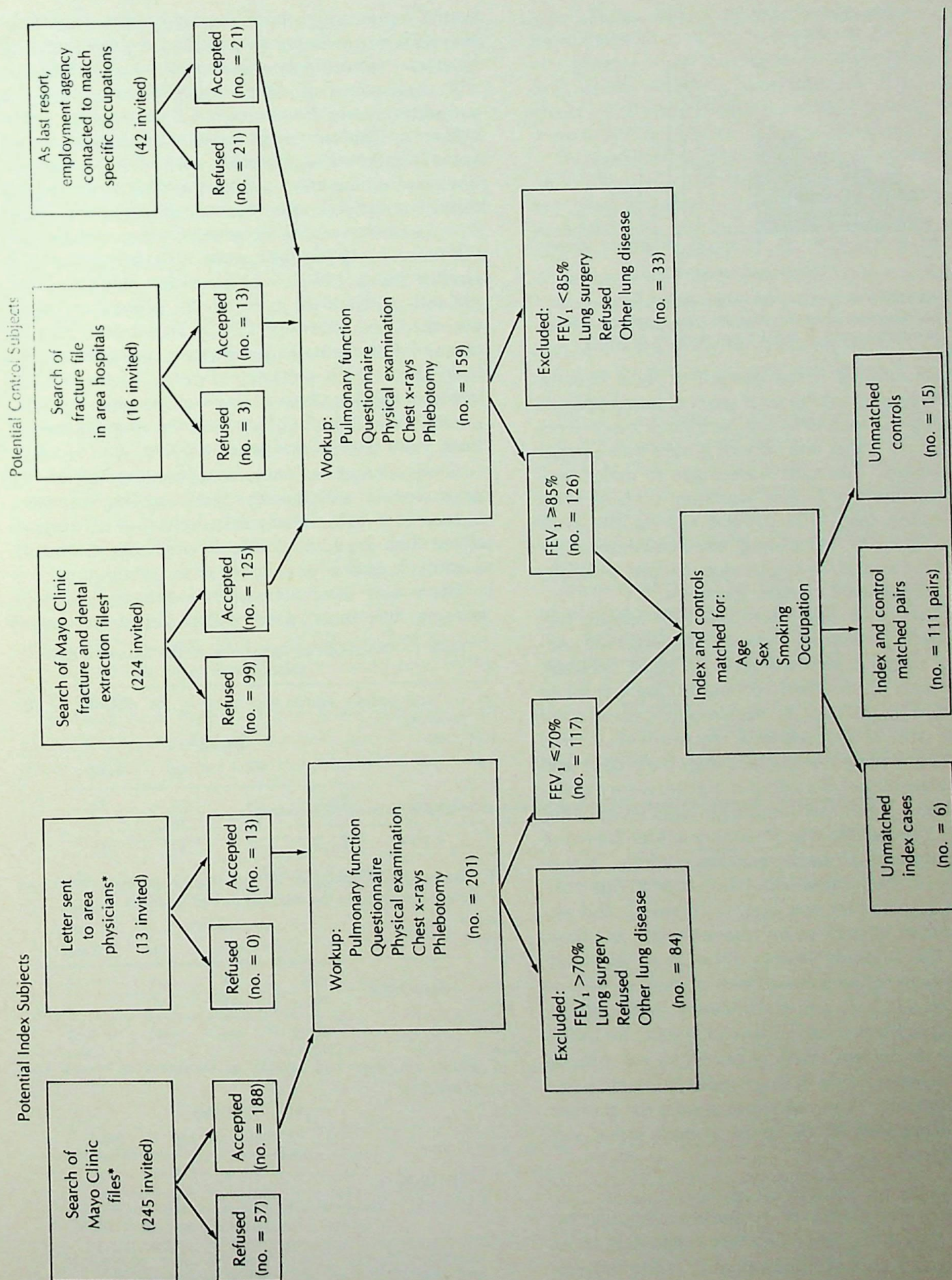
Ages were matched as closely as possible. The age range of all subjects was 45 to 60 years, and the mean age was 53.4 years for index and 51.9 years for control subjects for a mean difference in age of 1.5 years.

*Considered those with diagnoses of COPD, emphysema, chronic bronchitis, and asthmatic bronchitis excluding allergic asthma.
†Excluded those with diagnosis of allergic asthma.

Fig. 2. Schematic of subject selection and matching.

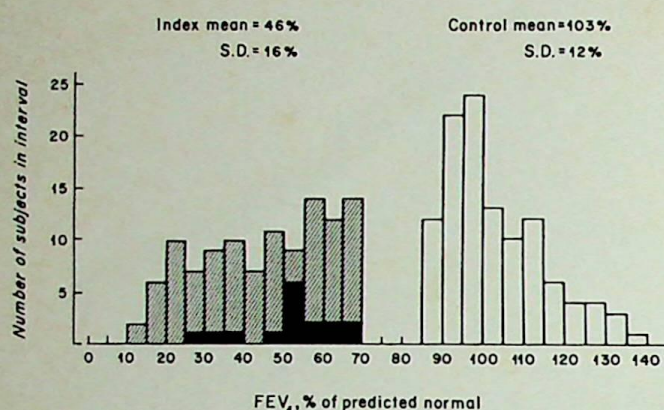



Fig. 3. Distribution of FEV_1 for index cases and controls. Clear bars represent controls; stippled bars represent index cases; solid bars represent index cases denying dyspnea.

Because farming is the dustiest of local occupations, we determined the total years of farm exposure in all subjects regardless of current primary work classification. Index and control groups had comparable exposure. Attempts were made to match specific occupations of longest duration, such as sheet metal working, truck driving, meat packing, and white collar occupations. We distinguished between indoor and outdoor aspects of similar occupations, acknowledging the seasonal climatic extremes.

Matching for smoking was based on relative lifelong cigarette pack-years (average daily cigarette consumption in packs times number of years smoked). Subjects with 0, less than 10, and 10 pack-years or more were classified as nonsmokers, moderate smokers, and heavy smokers, respectively. Nine matched pairs were nonsmokers (two male pairs and seven female pairs). Six additional pairs were moderate smokers. Ninety-six remaining pairs were heavy smokers. The median was 38 pack-years for the entire index group and 33 pack-years for controls. It was impossible in the limited population base to find controls that matched for sex, age, occupation, and absolute number of pack-years. Nevertheless, we have detailed the smoking history more than in many studies, where subjects have been classed merely as smokers, ex-smokers, and nonsmokers. Although the median of pack-years was somewhat greater for index cases than for controls, 62% of controls were current smokers, whereas only 40% of index subjects were current smokers. The statistical mode for current daily smoking was 20 cigarettes in both index and control groups.

RESULTS

Comparative Prevalence of Symptoms Obtained by Standard Questionnaire.—We anticipated that, in response to a standard questionnaire, index subjects would report a high frequency of cough, expectoration (phlegm), wheezing, whistling respirations, and dyspnea. Interestingly, the control subjects had significant degrees of cough and phlegm, but these symptoms were less frequent than among index subjects. The results of matched-pair analysis of some questionnaire responses are shown in Table 1. There were nine index subjects and six controls who denied cough but admitted to phlegm production. Attacks of dyspnea with wheeze were common in index cases (59%) but were also present in the control group (9%). Not all index subjects complained of dyspnea, even in the presence of pronounced reduction of FEV_1. This could not be explained on the basis of sedentary occupation; only four were office workers. Twelve of the 16 did inside or outside physical work or farmed. The FEV_1 among these index subjects denying dyspnea ranged from 39 to 67% of that predicted (Fig. 3).

Grades of dyspnea, compared between the matched pairs, were (as anticipated) significantly higher among index cases. This was particularly true of dyspnea worse than grade 1. Difficulty with chest illnesses was much greater among the index group.

There was similar frequency of reported hives, eczema, hay fever, nasal polyps, or sinus surgery

Table 1.—**Matched-Pair Responses on the Expanded NHLI Questionnaire**

A. Do you usually cough first thing in the morning in bad weather?

		INDEX*		
		Yes	No	Total
CONTROL	Yes	24	11	35
	No	50	26	76
	Total	74	37	111

B. Do you usually bring up phlegm, sputum, or mucus from your chest first thing in the morning in bad weather?

		INDEX*		
		Yes	No	Total
CONTROL	Yes	24	12	36
	No	45	29	74
	Total	69	41	110

C. Have you ever had attacks of shortness of breath with wheezing?

		INDEX*		
		Yes	No	Total
CONTROL	Yes	7	3	10
	No	59	42	101
	Total	66	45	111

*Significant difference from controls ($P<0.001$); two-tailed P value associated with χ^2 test of correlated proportions.

and "allergy." The types of allergy reported in both groups were similar and were ascribed to such factors as medicines, foods, various dusts, fumes, or pollens.

Pulmonary Function.—Although we used the FEV_1 to classify each subject as index or control, comparison of various values on the forced expiratory flow volume (FEFV) loop, as anticipated, also showed highly significant differences. Within the index and control male groups, there were highly significant (two-tailed test, $P<0.01$) correlations between FEV_1 and MEF_{25}, MEF_{50}, MEF_{75}, PEF, TLC by x-ray, and VC obtained from the flow-volume curve. All correlations were positive except for the one with TLC by x-ray in index cases. The values for correlations within the female groups were similar in magnitude to those within the male groups, but because of small numbers were not statistically significant.

Relationship of Smoking to Pulmonary Function Within Groups.—Correlation coefficients were determined for lifetime pack-years of cigarette smoking with age and various values of pulmonary function within each group of control males and females (Table 2). Significant correlations were detected for pack-years with FEV_1, MEF_{50}, and MEF_{75}, slope of the nitrogen plateau (phase III), and relative size of phase IV (closing volume) in the control male group, and with TLC and MEF_{75} in the control female group. No significant correlations were found in the index group.

α_1-Antitrypsin Types.—Three index subjects with severe COPD and PiZ phenotypes; two of these had never smoked. None of the controls had this phenotype. There were eight MZ phenotypes in the index group and five in the controls; both frequencies are greater than that seen in the general white population, which is approximately 2%. The frequencies of other phenotypes (no. index/no. control) were MM (95/97), MS (5/7), FM (0/1), and SS (0/1).

The endocrine portion of the questionnaire did not delineate any differences in the groups regarding the presence of acne, hirsutism, diabetes mellitus, or pituitary or thyroid disorder, and the menstrual histories were similar in the women. Blood levels of testosterone, estrogen, follicle-stimulating hormone, and luteinizing hormone were likewise normal in the two groups. Twenty-four-hour urinary excretions of hydroxyproline, a possible index of metabolic turnover of collagen, were not different in the two groups.[12]

Physical Findings.—Cardiopulmonary physical examination was carried out by two of us (R.D.M. or N.G.G.H.) without knowledge of the responses to the questionnaire. The family health pedigree was drawn up by the clinical geneticist who administered the questionnaire. No additional history was taken.

Intensity of breath sounds by lung region, presence of rales or wheezing during quiet and forced breathing, and heart sounds were noted. Abnormal breath sounds were noted in 58% of index subjects as compared with 2% of control subjects. Rales were present in 14% of index subjects and only in 1% of control subjects. Wheezing during quiet breathing was present in 14% of index subjects as compared

Table 2.—**Correlation Coefficients of Pack-Years With Age and Various Pulmonary Function Results**

	Control		Index	
Factor	Female (N = 20)	Male (N = 104)	Female (N = 22)	Male (N = 96)
Pack-years				
Mean	16.75	33.66	21.05	47.83
SD	14.72	19.48	17.51	27.52
	Correlations			
Age (yr)	−0.28	0.03	−0.26	0.11
FVC (L)	0.41	−0.17	0.05	−0.04
TLC x-ray (L)	0.65 (.002)*	0.002	0.13	0.12
FEV_1 (L)	0.15	−0.26 (.007)*	0.13	−0.05
PEF (L)	0.20	−0.03	0.03	0.06
MEF_{50} (L/s)	−0.29	−0.23 (.017)*	0.16	0.03
MEF_{75} (L/s)	−0.44 (.051)*	−0.29 (.003)*	0.24	0.12
Slope phase III†	0.26	0.36 (.001)*	. . .	. . .
CV/VC‡	0.02	0.34 (.001)*	. . .	. . .

*Significantly different from zero; P value within parentheses. P is the value associated with the two-tailed test of the population correlation coefficient equal to zero.

†Slope of phase III from single-breath N_2 curve (% change in N_2/liters expired); done only in controls.

‡Volume of phase IV from single-breath N_2 curve divided by VC obtained from same maneuver (%); done only in controls.

Table 3.—**Cardiopulmonary Physical Examination Results in 111 Index and Control Matched Pairs**

Measurement	Index			Control			Difference‡		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Height (cm)	172.3	7.8	148.0 to 188.0	173.7	7.1	150.0 to 190.0	−1.3	8.0	−26.0 to 20.0
Weight (kg)	73.8	14.2	41.0 to 122.0	79.5	13.4	50.0 to 127.0	−5.8*	16.4	−71.0 to 44.0
BP (sys) (mm Hg)	126.3	16.8	100.0 to 170.0	125.8	16.8	94.0 to 170.0	0.5	23.2	−55.0 to 55.0
BP (diast) (mm Hg)	81.6	9.4	60.0 to 110.0	81.8	9.0	65.0 to 110.0	−0.2	12.2	−35.0 to 35.0
Pulse (beats/min)	82.5	11.0	60.0 to 120.0	75.6	10.2	54.0 to 112.0	6.9†	14.5	−32.0 to 44.0
Subjective MMF (L/s)	0.7	0.4	0.2 to 2.0	2.2	0.9	0.8 to 6.0	−1.4†	1.0	−5.4 to 0.5
Expiratory flow over trachea (sec)	7.6	2.7	2.0 to 15.0	3.8	1.7	1.0 to 10.0	3.7†	2.8	−3.0 to 13.0
Diaphragmatic descent (cm)	4.1	1.3	0.0 to 7.0	6.1	1.2	2.5 to 9.0	−2.0†	1.6	−6.0 to 2.0

Statistically significant: *$P<0.0003$; †$P<0.0001$; two-tailed P value associated with paired t test.
‡Difference is the match paired difference, index-control.

with 0% of control subjects. Wheezing during forced breathing was noted for 87% of index subjects and only 26% of control subjects.

Comparisons of maximal excursion of each hemidiaphragm, time of audible expiratory air flow over the trachea measured in seconds, and a subjective estimate of the maximal midexpiratory flow (MMF) during the forced vital capacity effort are shown in Table 3. Weights were significantly lower in index subjects, reflecting the well-known tendency of loss of weight in the more advanced stages of COPD. Blood pressure was similar in the two groups, but the pulse rate was significantly more rapid in index subjects. Mean expiratory flow time over the trachea during FVC effort was significantly longer; the mean full diaphragmatic excursion was significantly less; and the examiner's subjective estimate of the MMF was significantly less in index subjects than in controls (Table 3).

DISCUSSION

The index and control subjects appeared to have been well matched for relative lifetime pack-years. Considering that controls had FEV_1 values of at least 85% of that predicted, it is interesting that in this control group a statistical correlation was found between pack-year exposure and several measures of flow on the maximal expiratory flow-volume loop as well as on the FEV_1. Within the 15-year cohort, there was a positive but not statistically significant correlation between pack-years and age in the index and control male groups. Among the female groups, there was a negative but not significant correlation between age and pack-years. This indicates that older women may have fewer pack-years than do younger women. The generally lower prevalence of COPD among women has been ascribed to their lower cigarette consumption. However, the lifelong smoking exposure in our female group with established COPD was significantly less than that in index male groups. Seven women (1 a PiZ phenotype) among 20 were nonsmokers, while only 2 men (1 a PiZ phenotype) among 91 were nonsmokers. Thus, while women formed only 18% of our index group, their lesser smoking suggests that there are important pathogenic factors other than smoking or that women are less tolerant to smoking. If the tolerance to smoking were similar and smoking were the overriding risk factor in both men and women, one would have expected that the women with established COPD in our group would have smoking histories similar to those of the men. The mean FEV_1 in index women was 48% of that predicted and in index men, 45% of that predicted. Past respiratory infection was not more frequent in index women than in men. Ratios of males to females were similar to those of earlier studies summarized by Webster and associates.[13]

Among the 111 controls, 19 (17%) had large closing volumes and 18 (16%) had steeper-than-normal slopes of the alveolar nitrogen plateau (phase III). The proportion and degree of these abnormalities of the single-breath nitrogen test were not as high as those reported by Buist and Ross,[9,10] when subjects were screened, regardless of their FEV_1 values. The positive correlation of lifelong pack-year cigarette smoking with slope of phase III and closing volume further supports the speculation that smoking contributes to early airway disease in asymptomatic subjects with otherwise normal pulmonary function (Table 2).

The questionnaire highlighted some well-known characteristics of patients with COPD. They had higher incidence of respiratory infections by several criteria: past diagnoses of pneumonia and pleurisy, number of hospitalizations, and total number of all

respiratory infections in recent years. Although these factors may not have initiated the COPD, they likely contributed to the pathogenesis.

Problems with the upper airways, polyps, past sinus surgery, and any type of apparent allergy were not more frequent in the index subjects than in the controls. Persons with seasonal respiratory symptoms were excluded by design. It is of interest that 42 index subjects and an identical number of controls reported being allergic to at least one substance, most of which were medicines and not the usual allergens ascribed to asthmatic patients, who were excluded from this study.

Standard respiratory questionnaires, although better than unstructured clinical histories in collecting subjective information on population groups, have shortcomings, especially when used to arrive at a clinical diagnosis such as chronic bronchitis.[14,15] Actual pulmonary function tests have been advocated as being more reliable, especially if chronic bronchitis is used as an indication of the type of COPD.[14] Using preset limits for flow rates, we sharply separated our two groups. When we use the questionnaire criteria of cough and phlegm, which are traditionally accepted for indicating chronic bronchitis, there is considerable overlap between the two groups. For example, we found that 32 control subjects responded positively to the questions regarding phlegm being present at least 3 months of every year—criteria for a diagnosis of chronic bronchitis established by reports from the British Medical Research Council.

Another problem with the questionnaire is related to the patient who denies, seemingly sincerely, symptoms that really are present. Sixteen index subjects who denied dyspnea actually had severe reduction of maximal flow rates (Fig. 3). This lack of association between reported dyspnea and measures of pulmonary function has been reported many times and merely documents further the sometimes poor association between subjective measure of dyspnea and any single measure of pulmonary function.[16]

Review of results of the physical examination of all our subjects showed that the estimated values for MMF between the two groups were significantly different even though there was considerable overlap. The duration of audible expiratory flow time heard over the trachea seems to add significantly to the assessment of ventilatory function. The descent of the diaphragm during full inspiration, as determined by percussion, showed significant differences between the two groups, and the descent is often reduced when maximal flow rates are greatly reduced. The total expiration time, best heard over the upper trachea, is the best measure of diffuse intrathoracic airway obstruction,[17] but is not commonly described in textbooks on physical diagnosis or chest disease.[18]

Assessment of the many laboratory variables obtained is under way to characterize more fully additional differences between the matched pairs with and without COPD.

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Protein Metabolism in the Black Bear Before and During Hibernation

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During 3 to 5 months of hibernation, the American black bear does not defecate, urinate, or require food or water. Although the bear loses 15 to 25% of its body weight during this period, there is no significant change in its lean body mass. No net accumulation of the usual nitrogenous products of protein catabolism can be demonstrated in the dormant bear, and there is a decrease in urea production during hibernation. Because of these findings, it has been hypothesized that the black bear can alter its protein metabolism during hibernation by some unknown mechanism. During this study, the metabolic rate of protein turnover in four adult male black bears was measured before, during, and after hibernation, using ^{125}I-labeled serum albumin from black bears as an indicator protein and ^{14}C-labeled leucine as an indicator amino acid. For albumin during both phases, the disappearance rate of labeled albumin from serum was measured over 2 weeks and its turnover rate was calculated from these data. For [^{14}C]leucine, the amino acid was injected during and after hibernation and its appearance in total proteins of plasma was measured. The results using labeled albumin revealed a threefold to fivefold increase in turnover of protein during hibernation compared with protein turnover before hibernation. Leucine data supported these findings; more labeled leucine was incorporated in plasma proteins during hibernation than in the active state in spring. There were no significant changes in hematocrit, serum albumin concentration, thyroxine, or thyroxine-binding globulin between active and dormant periods, although triiodothyronine tended to decrease during hibernation. We speculate that increased protein turnover suggests a strongly acting protein-anabolic mechanism that would tend to compete with other catabolic pathways for amino acids. Another consequence of this increased protein turnover would be thermogenesis. This may have helped prevent any undue decrease in body temperature. It is notable that the body temperature of the dormant bear is appreciably higher than that of other hibernating animals.

The American black bear (Ursus americanus), because of its unusual metabolic adaptations during hibernation, is a unique animal model for the study of protein metabolism in mammals. Unlike those animals that are deep hibernators, the bear remains fully arousable and capable of defending itself throughout its entire period of winter sleep.[1-4] Also, in contrast to its deep-sleeping mammalian relatives, the bear does not have the decrease in metabolism or the profound hypothermia so typical of the hibernating state.[2,5-7] Although the bear is not a classic hibernator, it is capable of sleeping continuously for 3 to 5 months during the winter and is able to do so without the need to eat, drink, defecate, or urinate.[1-5]

The results of some recent studies of ursine physiology in our laboratory present evidence that the bear achieves these metabolic adaptations by altering its protein metabolism during hibernation through some unknown mechanism. We were able to demonstrate that, although the bear loses from 15 to 25% of its body weight during hibernation, there is no significant change in its lean body mass during the same period.[1,2,8,9]

This work was supported in part by Northwest Area Foundation Grant SPF 8469.

Table 1.—**Mass Measurements in Bears Before and During Hibernation**

	Bear 1		Bear 2		Bear 3		Bear 4	
	Before	During	Before	During	Before	During	Before	During
Total body mass (kg)	199.57	180.45	147.27	133.64	162.73	141.82	133.18	94.55*
Total body water (liters)	74.25	76.65	61.67	71.91	66.05	57.58	68.60	
Lean body mass (kg)	101.43	104.71	84.25	98.24	90.23	78.66	93.72	
Fat body mass (kg)	98.14	75.74	63.02	35.40	72.50	63.16	39.46	

*Value was obtained after death.

Moreover, even though the bear does not defecate or urinate for months at a time during hibernation, there is no net accumulation of the usual nitrogenous products of protein catabolism such as oligopeptides, amino acids, ammonia, uric acid, or urea in the blood, feces, or urine of the dormant bear. In fact, the production of urea actually decreases significantly during hibernation.[10] If no catabolic products of protein metabolism are formed that require urinary excretion, and if the water of metabolism equals the insensible water loss in a sleeping bear that is neither eating nor drinking, then there is no physiologic need for the bear to defecate or urinate during hibernation.

Other experimental evidence shows that there is a definite decrease in the respiratory quotient from 0.78 to 0.60 during hibernation when the bear is starving and a concomitant increase in serum lipids including cholesterol, phospholipids, and triglycerides.[1]

These findings strongly support our hypothesis that, during hibernation, the black bear achieves independence from food and water by acquiring its metabolic energy primarily from catabolizing stored lipids and that a significant decrease in protein catabolism, as evidenced by the constancy of lean body mass and the decrease in urea production, appears to be the most important means by which the state of hibernation is achieved and maintained.[1,2,10]

The purpose of this study was to measure the metabolic turnover of protein in the black bear before, during, and after hibernation, using ^{125}I-labeled serum albumin from black bears as an indicator protein and ^{14}C-labeled leucine as an indicator amino acid to attempt to correlate the data obtained with the findings from our earlier studies.

METHODS

The animals studied during these experiments were four adult male American black bears provided by the Institute Hills Farm of Mayo Foundation. Each of the bears was in good health at the onset of both studies, and the same four bears that were studied before hibernation were studied again during and after hibernation so that each bear could be observed individually for any metabolic change that might occur during the period of study. When it was necessary to immobilize the bears, phencyclidine hydrochloride and promazine hydrochloride were injected intramuscularly.[11]

Before beginning these experiments, it was necessary to obtain biochemically and immunologically unaltered serum albumin from black bears, pure enough to be nonantigenic in the experimental animals. Serum was taken from each of the four bears, pooled, and sent to Microbiological Associates, Walkersville, Maryland, where albumin was isolated using Sephadex G-200 and carboxymethyl cellulose chromatography. The purity of the black-bear albumin was documented by serum protein electrophoresis and immunoelectrophoresis, which showed that 97% was albumin and 3% nonalbumin protein.

Once the purified black-bear albumin had been obtained, it was labeled with iodine-125 by oxidation of [^{125}I]sodium iodide with sodium hypochlorite at the Squibb Institute for Medicine Research, New Brunswick, New Jersey. The efficiency of the labeling technique was assessed by subjecting the labeled albumin to thin-layer chromatography and then auto-

Table 2.—**Hematocrit and Albumin Determinations in Bears Before and During Hibernation**

	Bear 1		Bear 2		Bear 3		Bear 4	
	Before	During	Before	During	Before	During	Before	During
Hematocrit (%)	45.20	50.40	45.40	44.20	42.20	50.10	52.00	
Albumin (g/dl)	4.24	4.19	4.01	4.19	3.64	4.28	3.51	

Table 3.—**Thyroid Hormone and Thyroxine-Binding Globulin Concentrations in Bears Before and During Hibernation**

	Bear 1		Bear 2		Bear 3		Bear 4	
	Before	During	Before	During	Before	During	Before	During
Total thyroxine (μg/dl)	0.9	2.0	0.5	2.4	1.0	2.4	1.0	
Total triiodothyronine (ng/dl)	80	27	62	47	66	63	61	
Thyroxine-binding globulin (μg/dl)	18.4	16.1	15.4	12.9	13.6	15.5	16.9	

radiography, and also by precipitating the labeled albumin with 10% trichloroacetic acid and measuring the precipitate radioactivity with a well-type scintillation counter. In the active study, 91.4% of the iodine was bound to albumin and, in the hibernation study, 92.2% of the iodine was bound to albumin.

During the experiment before hibernation, each of the four bears was studied for 2 weeks and the following procedures were performed. On the first day, each bear was immobilized, tagged with an identification number, and weighed. A urinary catheter was inserted and urine was collected every 4 hours for the first 24 hours of the experiment. Blood was removed with a syringe from a femoral vein (as were all blood samples) and the following determinations were made: hematocrit, serum albumin concentrations, total thyroxine, total triiodothyronine, and thyroxine-binding globulin.

After the blood was drawn, 50 g of deuterium oxide and 150 μCi of ^{125}I-labeled black-bear albumin were injected through the same needle in the femoral vein. Four hours after the injection of the deuterium oxide, a blood sample was drawn to determine total body water by the method of Schloerb and associates.[12] Then, from this value, both lean body mass and total body fat were calculated as described by Pace and Rathbun.[13]

To determine the disappearance rate of [^{125}I]albumin from the peripheral circulation of each bear, blood samples were taken at 10, 20, and 30 minutes and 1, 2, 4, 8, 12, and 24 hours after the initial injection of the labeled albumin. The labeled albumin in each of these samples was then precipitated with 10% trichloroacetic acid, the radioactivity of the precipitate was measured in a well counter, and the half-life of the albumin turnover was determined by the method described by Sterling.[14] The volume of urine obtained during the first 24 hours of the study was measured and its radioactivity was determined.

After the first 24 hours, the bears were permitted to recover from anesthesia. Then, once every 48 hours for the next 10 days, the bears were immobilized just long enough to obtain blood samples for measuring the concentration of albumin and the disappearance rate of [^{125}I]albumin by the method described above.

On the last day of the study before hibernation, all of the procedures described for the first day were repeated except for the injection of [^{125}I]albumin. The four bears were then permitted to go into hibernation spontaneously. They had been dormant for at least 6 weeks before the second experiment was begun.

The experiment during hibernation was also of 2 weeks' duration and the protocol was identical to that followed before hibernation except that no urine was collected because the bears did not urinate during hibernation. Also, on the first day of the study with the dormant bears, additional blood samples were drawn from each bear to measure the amount of background radioactivity remaining from the experiment before hibernation and, on the last day of the study during dormancy, additional blood samples were obtained to measure blood urea levels in each of the bears.

One month later, while the bears were still in hibernation, 100 to 125 μCi of uniformly labeled [^{14}C]leucine were injected as a bolus into the femoral vein of each bear and timed collections of femoral venous blood from the opposite vein were obtained over the next 24 hours, with additional samples taken during the next 6 to 8 days. The study

Table 4.—**Albumin Turnover Half-Life in Bears Before and During Hibernation**

	Bear 1		Bear 2		Bear 3		Bear 4	
	Before	During	Before	During	Before	During	Before	During
Albumin turnover half-life (days)	8.33	2.77	9.04	1.70	7.04	2.51	7.60	
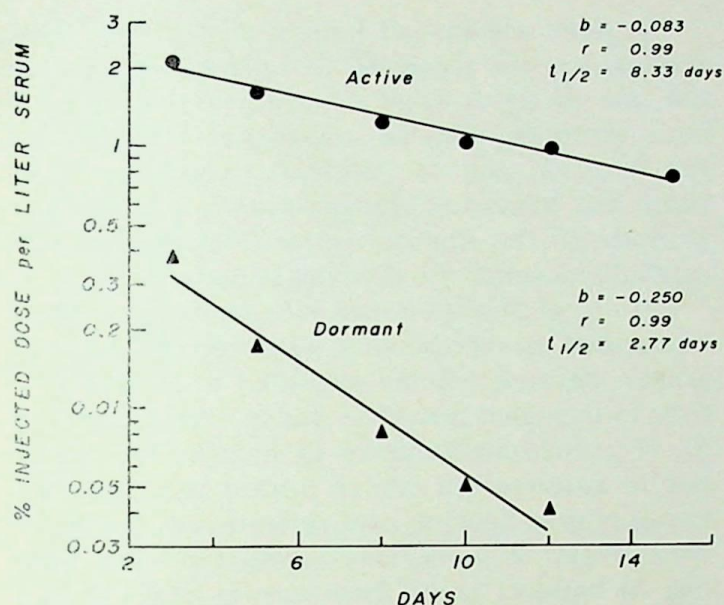
Fig. 1. Disappearance of [125I]albumin from serum in bear 1.

was repeated during the following spring, after the bears had been active for 2 months. At that time, each bear received the same dose of labeled leucine. (Anesthesia and handling of the bears were the same as in the albumin experiments.) In all blood samples, plasma proteins were precipitated and washed three times with 5% trichloroacetic acid and three times with methanol and ether. They were dried and weighed and a measured quantity was counted in a Packard tricarb-liquid scintillator model 314EX. A portion of the precipitate was acid hydrolyzed and those amino acids containing activity were determined in a Beckman model 120B amino acid analyzer linked to a Beckman LS-100L liquid scintillation counter.

Before and after hibernation, the bears were housed in open-air cages at the Institute Hills Farm where they were exposed to ambient temperatures, natural photoperiodism, and natural variations of wind and moisture, although they had enclosed shelters within their cages. They were fed water and dry dog food (ad libitum) which contained 23% protein and 8% fat. The noise level was that of a typical Minnesota farm.

During hibernation, the bears were housed within an abandoned root cellar dug in the side of a hill and lined with concrete. They were continually in the dark and sheltered from the cold, wind, and moisture. They slept on beds of straw in artificial dens made from steel culverts. No food or water was available to the bears during hibernation. The root cellar was insulated enough so that the bears were protected from all noises except those they made as they slept.

RESULTS

During the second week of the 6-week period between the two studies, when the bears were entering the state of hibernation and were adapting metabolically to it, bear 4 was found dead in its culvert den. The bear had lost 30% of its body weight in less than 2 weeks (Table 1), had urinated on its bed of straw, and a postmortem blood sample contained 650 mg/dl urea. Obviously, bear 4 had failed to hibernate. The findings were similar to those shown by black bears when starved during the summer months, when they are normally quite active.[10] In our experience, bears will not hibernate if aroused frequently in their dens in winter. In order to allow them to enter hibernation, they are left undisturbed most of the time and usually observed only at 2-week intervals. Bear 4, unfortunately, failed to hibernate and died. Bears, however, die in the wilds from unknown causes during the winter.[15] Our experience suggests they may have failed to achieve the state of hibernation.

In the other three bears, the losses in body weight during hibernation were within the range reported in previous studies.[1,10,11] There was no change in
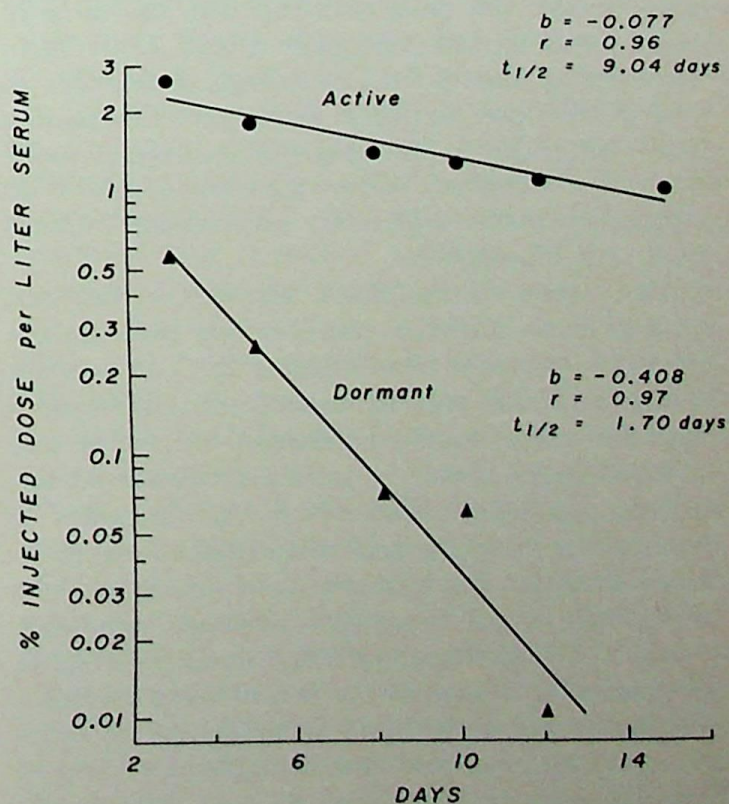
Fig. 2. Disappearance of [125I]albumin from serum in bear 2.
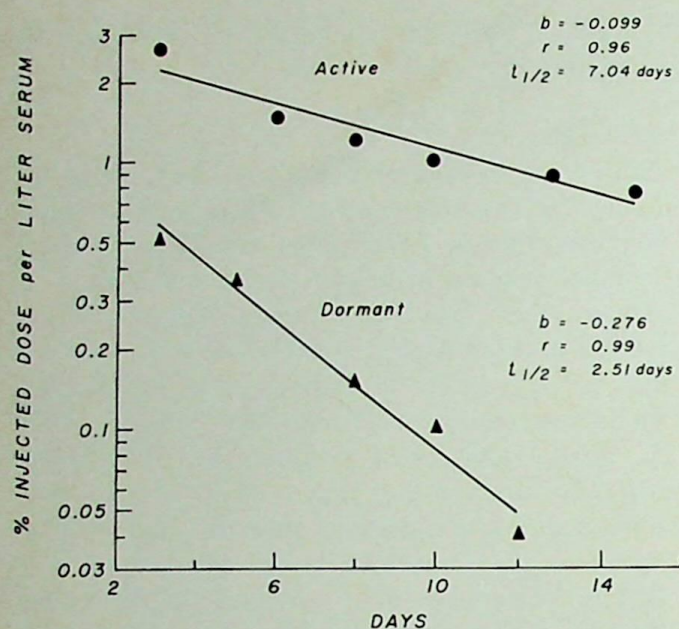
Fig. 3. Disappearance of [¹²⁵I]albumin from serum in bear 3.

total body water before and during hibernation in bear 1. These data supported earlier observations that total body water does not change during hibernation.[10] However, in bear 2, total body water increased and in bear 3 it decreased during hibernation. These variations may reflect the range of the method. In our previous experience, such wide differences have not been noted. An error in tabulating results may have occurred with the value of 57.58 liters representing bear 2 and 71.91 liters representing bear 3. If this is true, differences in total body water between active and hibernating states would be in the range of the experimental error for the method. Other than this, we have no explanation for the total body water results in bears 2 and 3 in hibernation.

There were no significant changes in hematocrit and mean albumin concentration between the active and dormant phases (Table 2). The increase in total thyroxine and the decrease in total triiodothyronine concentrations between the active and dormant studies (Table 3) were not considered statistically significant because of the low accuracy of the assay for thyroxine and triiodothyronine at these concentrations. The changes were similar to earlier findings in our laboratory although somewhat greater.[1] Triiodothyronine may actually decrease in hibernation. The thyroxine-binding globulin concentrations were found to be similar to those found in humans and the changes observed in these concentrations between the two studies were minimal and statistically insignificant (Table 3).

The most unexpected finding revealed by these studies was the threefold to fivefold increase in the rate of turnover of albumin in the dormant bears compared with the active bears (Table 4 and Fig. 1, 2, 3, and 4). Without exception, those bears that hibernated demonstrated a considerable increase in the rate at which [¹²⁵I]albumin was catabolized during the dormant period.

Results of studies using labeled leucine were interpreted as representing increased synthesis of protein because leucine appeared in plasma proteins in increased quantities during hibernation (Fig. 5). The increased uptake of leucine had to be due to an increased rate of protein anabolism because plasma leucine and protein concentrations were similar in winter and spring and, during the first 24 hours of study, there was no intake of food by the active bears that could have affected the specific activity of leucine. The fact that the specific activity of leucine remained elevated during the subsequent 8 days of study was interpreted by us as a function of rapid reentry of leucine back into plasma proteins after the protein had been catabolized. All of the activity of the plasma protein was found in leucine after acid hydrolysis.

DISCUSSION

Because catabolism of albumin is increased measurably during hibernation and because the serum albumin concentration remains constant during the same period, then the rate of albumin synthesis must equal that of albumin degradation. It follows that albumin synthesis is increased during hibernation. This conclusion was supported by the leucine data because, in hibernation, leucine entered plasma proteins in increased quantities. Since total proteins and leucine concentrations of plasma remained constant, there must have been an increase in turnover of protein to account for the increased uptake of labeled leucine. Thus, these findings supported those obtained from the albumin study indicating that there is increased turnover of plasma proteins in hibernation. Be-
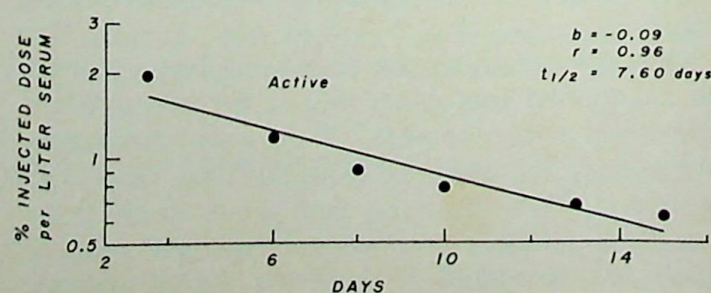
Fig. 4. Disappearance of [¹²⁵I]albumin from serum in bear 4.
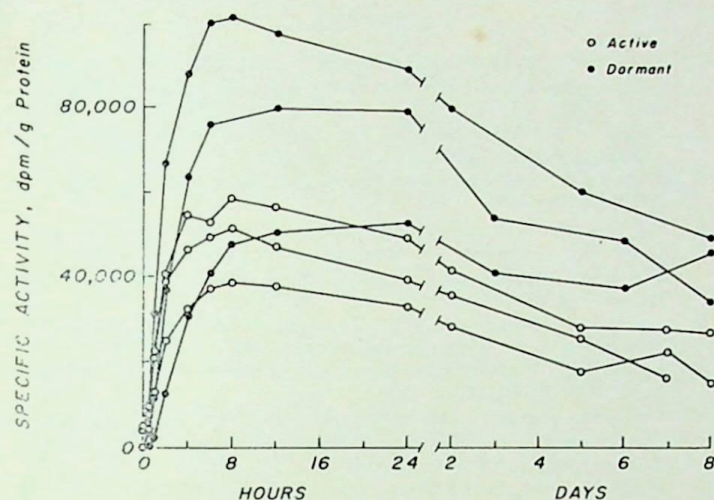
Fig. 5. ^{14}C activity in plasma proteins in active and dormant states after injection of [^{14}C]leucine.

cause albumin and plasma proteins are synthesized by the liver, this organ must undergo two distinct changes in the hibernating bear, as judged from these data. The liver appears to increase its rate of protein synthesis while decreasing its rate of urea production.[10] Our original hypothesis was that, concomitant with a decrease in urea synthesis, a decrease in protein turnover might also occur.[1] The data showed the opposite to occur when albumin and leucine were the models used to study protein metabolism.

Because of this unexpected finding of increased protein turnover in hibernation, we evaluated our methodology critically. Several problems could lead to a falsely high turnover of albumin in a comparative study such as this. (1) Different specific activities of the injected albumin might lead to a difference in early disappearance but should have little influence on the slope after mixing has occurred. (2) Different quality of the protein iodination or different quality of the albumin itself could produce difficulties. In our study, labeling procedures were performed in the same laboratory and the same batch of albumin was used in both procedures; specific activity was also comparable. (3) Free iodine, because of its shorter biologic half-life, would also lead to a falsely high turnover rate, particularly in the first few days. Our quality control procedures, however, showed that the amount of free iodine at the time of injection did not exceed 8% and was similar in both studies. (4) Protein loss through the gastrointestinal tract or in urine is another possible cause of inconsistent results. There was measurable protein in the urine of the bears when tested before hibernation, but no urine or fecal loss was recognized during hibernation. Proteinuria may indeed have resulted in a faster-than-normal turnover of albumin in the bear before hibernation, but this cannot explain the difference between the two studies.

Lastly, one could conceive that, in hibernation, a different method of handling albumin exists so that the mathematical model used for the interpretation of normal data is not applicable. However, significant changes in albumin metabolism do occur in hibernation and the concept of slow metabolism in winter sleep is probably too simplistic, no matter how attractive it may be. Furthermore, the labeled leucine studies supported the albumin findings and the leucine studies were done in the same winter, on the same bears, and then repeated in spring. And protein turnover was found by this method also to be increased in winter from fall values and then decreased once more in spring.

Just why protein metabolism is increased during hibernation is open to speculation. Perhaps the increased rate of synthesis of albumin and plasma proteins is a mechanism that prevents compounds such as essential amino acids, like leucine, which are required for protein synthesis, from being catabolized into carbon dioxide, water, and urea. If essential amino acids were broken down to any degree, protein synthesis would be impaired and lean body mass would decrease. This does not occur in hibernating bears. Such an adaptive mechanism has been described in protein-and-calorie-deficient rats—essential amino acids are incorporated at increased rates into liver proteins whereas their oxidative degradation is markedly decreased.[16] The increased rate of protein synthesis in the hibernating bear might represent a similar adaptive mechanism. Essential carbon skeletons are preserved when their turnover rates are increased in protein metabolism and their entry into the urea cycle is decreased. Such reciprocal changes are not unique to the hibernating bear. Usually, in mammalian tissues, a change in one route of disposal of amino acids is accompanied by a reciprocal change in others.[17] There are three metabolic outlets for amino acids in the body: protein synthesis; synthesis of compounds of low molecular weight such as creatine and nonessential amino acids; and degradation through pathways of amino acid catabolism.[17] In hibernating bears, protein synthesis increases and amino acid catabolism decreases. An analogous situation is found in the newborn animal, which

shows intense protein synthesis but limited degradation of circulating amino acids.[17]

Increased protein synthesis, besides preserving essential amino acids from catabolism, is also needed for the increased demands for enzymes necessary for the vitality of the bear as it adapts to conditions demanded by hibernation. Surely there must be an increased synthesis of lipolytic and gluconeogenic enzymes during hibernation because the bear relies mostly on fat as an energy source while preserving constant levels of blood glucose and alanine,[1] a feature not found in starving human beings.[18] Also, protein synthesis is required to provide the proteolytic and synthetic enzymes responsible for the apparently increased protein turnover in the dormant bear.

A byproduct of increased protein turnover is thermogenesis. The heat produced during protein metabolism in the hibernating bear may be partly responsible for preventing the profound drop in body temperature during hibernation that is a common feature of the classic deep hibernators.[6]

The mechanism whereby the black bear is capable of altering its protein metabolism during hibernation is unknown; however, unique metabolic adaptations do occur, as evidenced by this and other studies. A better understanding of the physiology of the black bear may contribute significantly to the management of anephric human patients or those in chronic renal failure, to the ultimate control of human obesity, and to further comprehension of the complex process of aging.

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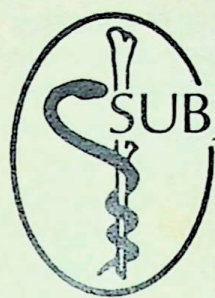
SUBJECT REVIEW
Autologous Blood Transfusion

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Autologous blood transfusion is a procedure in which blood is removed from a donor and returned to his circulation at some later time. Autologous transfusion can be performed in three ways: (1) preoperative blood collection, storage, and retransfusion during surgery; (2) immediate preoperative phlebotomy with subsequent artificial hemodilution and later return of the phlebotomized blood; and (3) intraoperative blood salvage and retransfusion. All three methods of autologous transfusion offer a potentially superior method of blood transfusion which eliminates many of the problems and complications associated with the banking and administration of homologous donor blood.

On Sept. 10, 1974, the Secretary of Health, Education and Welfare published a National Blood Policy[1] in the *Federal Register*. This policy set forth four principal goals: an adequate blood supply, availability to all people, efficient utilization, and the highest standards of transfusion therapy with the safest possible blood.

The newly formed American Blood Commission, with the support of the blood-banking community and professional and consumer groups, has as its goal the implementation of this policy. Undoubtedly the Commission will call for reappraisal of current practices and introduction of new ones. Among these new practices, we feel, should be a greatly expanded use of autologous blood transfusion to help meet many of the goals of the National Blood Policy. Autologous transfusion would provide the recipient with the safest and best possible blood product—his own blood. It is a potentially large new source of blood, and it can provide a supply of blood in many rural areas that are now dependent on large urban centers.

Because of the potential value of autologous transfusion and the impetus provided by the National Blood Policy and the American Blood Commission, we have undertaken an extensive review of autologous blood transfusion to determine the progress made in this field and to identify problems that need further investigation. This review has made the potential value of autologous blood transfusion even more apparent to us and has stimulated plans to increase its use within our institution.

Autologous transfusion is a procedure in which blood is removed from a donor and returned—sooner or later—to his circulation. This procedure was described as early as 1818 and has been used sporadically since then.[2] Its use was originally advocated as an emergency measure to combat sudden massive hemorrhage in the absence of available donor blood. Early reports indicated that it was an effective and frequently life-saving means of transfusion. Recent publications have confirmed these early successes and suggest a more widespread use of autologous transfusion.

Autologous transfusion can be performed in three ways: (1) preoperative* blood collection, storage, and retransfusion during or after surgery; (2) immediate preoperative phlebotomy, artificial hemodilution, and postoperative return of the phlebotomized blood; and (3) intraoperative* blood salvage and retransfusion. All three methods offer the following advantages over the use of homologous donor blood:

1. No transmission of disease (hepatitis, syphilis, malaria, cytomegalovirus, Epstein-Barr virus[3-5]).
2. No risk of isoimmunization to erythrocyte, leukocyte, platelet, or protein antigens.[6-8]
3. No risk of hemolytic, febrile, allergic, or graft versus host reactions.[6-8]
4. Ready availability of blood for patients for whom compatible blood is unavailable.[9,10]
5. Elimination of many technical errors of typing and crossmatching which can lead to incompatible transfusions.[11]
6. Provision of a new source of homologous blood when not needed for autologous use.[12,13]

PREOPERATIVE AUTOLOGOUS TRANSFUSION

Blood collected by preoperative phlebotomy can be stored as a liquid at 1 to 6°C or as frozen red cells for use during a subsequent routine surgical procedure. If the patient's hemoglobin level, cardiac status, and general condition permit, a series of preoperative phlebotomies can remove several (generally 4) units of blood for storage at 1 to 6°C.[14,15] Amounts in excess of 4 units can be collected over a more prolonged period and stored as frozen red cells. Long-term storage of frozen autologous red cells is especially valuable for the patient for whom compatible blood is difficult to find.

Storage at 1 to 6°C.—Preoperative phlebotomy and storage at 1 to 6°C of autologous blood have been in use since 1921, when Grant[16] used autologous transfusion for a patient with a rare blood type who had a cerebellar tumor. A phlebotomy was performed 24 hours before operation; the blood was stored in sodium citrate and reinfused after the surgery. Milles and associates[17] and Langston and associates[18] have used preoperative autologous transfusion since the early 1960's for more than 1,000 patients, successfully meeting more than 90% of their surgical blood needs. Others also have contributed to the development of this method. Newman and associates[19] recommended the use of iron therapy to increase the number of units that could be drawn preoperatively. Corpe and Liang[12] and Smith and Michael[13] have advocated the release of unused autologous blood for homologous use. Ascari and associates[20] recommended a "leapfrog" technique, by which 3—and as many as 6—units of a patient's blood could be obtained before surgery with none being older than 10 to 14 days. Lubin and associates[21] employed a similar technique and obtained several units of fresh frozen plasma as well.

In view of the many advantages of autologous transfusion, why has this form of transfusion therapy not gained wider acceptance and use? The extraordinary medical problems created by World War II gave impetus to the development of many large civilian blood banks primarily concerned and subsequently preoccupied with the collection, storage, and use of homologous donor blood. Other reasons for lack of interest in this form of transfusion include the facts that (1) autologous blood cannot be stored in a liquid state for more than 21 days; (2) frozen red cells are significantly more expensive for the patient and are unavailable in many areas; (3) successful use of autologous transfusion programs has not been widely publicized; (4) physicians fear the supposedly debilitating effect of phlebotomy on patients going to surgery; and (5) improper identification of an autologous unit could lead to disastrous consequences if the unit was administered to the wrong patient—necessitating caution to ensure that the autologous donor receives his own blood.[15,22]

Effects of Preoperative Phlebotomy.—The major concern voiced by most physicians is that preoperative phlebotomy will make the patient less fit for the stress of surgery. Over the past several years, investigations have revealed that patients easily tolerate preoperative withdrawal of 1 to 3 units.[13,17,21,23-28] A few have shown that as many as 4 to 8 units can be removed from an individual within 20 days before surgery without ill effect, provided that iron supplement is given.[19,29] These conclusions are based primarily on two assumptions: that phlebotomy is an adequate stimulus for increased marrow production of red cells, and that the plasma volume is regenerated rapidly after phlebotomy.

In patients with intact bone marrow and erythropoietin systems, the marrow rapidly replenishes red cells lost after hemorrhage. Marrow reticulocytes can be delivered to the circulation as early as 6 to 12 hours after the initial phlebotomy; but a full level of

*In some instances the words "preoperative" and "intraoperative" may not apply, although similar techniques are employed. For example, blood may be collected from patients with rare blood types even though surgery is not contemplated; or blood may be salvaged from traumatic wounds (for example, hemothorax) rather than from a surgical wound.

marrow production, releasing mature red cells into the circulation, usually is not reached until 8 to 10 days after the initial phlebotomy.[30-33] Hillman and Henderson[33] have shown that with oral iron administration the marrow can increase production to two or three times normal in response to blood loss. Others have shown that with iron supplement the response can be maintained over several weeks despite repeated phlebotomies. Finch and associates,[34] in 1950, studied patients from whom 500 ml was taken weekly, with and without oral iron supplement. In those undergoing weekly phlebotomy without iron, the hematocrit exhibited a continuous decrease from 44% to 33% over an 8-week period, whereas in those who received iron, it decreased from 44% to 37% during the first 5 weeks but then stabilized and subsequently increased slightly to 38% by the eighth week. Coleman and associates[35] later duplicated this work in a study of patients bled 500 to 1,000 ml/week.

These results imply additional benefits of preoperative autologous transfusion. Patients subjected to preoperative phlebotomy will arrive at surgery in a state of maximal hematopoiesis, whereas those who do not undergo preoperative phlebotomy will require several days before red-cell production reaches a maximal level, since marrow stimulation does not begin until the surgical blood loss occurs. In addition, it is to be presumed that many of the transfused autologous red cells will be younger and so will survive longer after reinfusion than transfused homologous red cells.

During the first 2 weeks after phlebotomy, the rate of marrow production can be increased to 3 or 4 times normal by parenteral iron therapy—though it returns to 2 to 3 times normal thereafter.[33,36,37] However, the dangers of parenteral iron make this form of iron supplementation undesirable.[38-41] Several investigators have shown oral iron to be as adequate as parenteral iron in treatment of blood-loss anemia.[29,35,42,43]

After phlebotomy, the plasma volume is replenished much more rapidly than the red-cell volume. Usually it returns to normal within 72 hours, if not sooner.[44-48] This is due to rapid mobilization of extravascular fluid and protein, immediate increase of protein (albumin) production, and a strong renin-angiotensin response.[49,50]

Investigations of Preoperative Autologous Transfusion.—In 1962 Milles and associates[51] reported their experience with a series of 53 patients whose ages ranged from 20 to 74 years. Those patients generally underwent one or two phlebotomies 4 or 5 days before surgery for chronic pulmonary tuberculosis (thoracoplasty, lobectomy, pneumonectomy). Criteria for selection as an autologous donor were similar to those recently suggested by the American Association of Blood Banks—that is, hemoglobin 11 g/dl or greater or hematocrit 34% or greater.[14,15] In an earlier paper, Langston and associates[52] reported various measurements made in 27 of 113 cases of autologous transfusion. The 27 patients donated 1 unit of blood several days before surgery and were not given iron. When they came for surgery they had lost an average of 1.0 g/dl of hemoglobin and had a decrease of 5% in hematocrit. The net loss for the group in red-cell volume was 155 ml, and the average gain in plasma volume was 366 ml. The average increase of reticulocytes in these patients 24 hours post phlebotomy was 0.6 to 0.7%. From the total of 113 patients, 154 units of blood were obtained; and these fulfilled 62% of all blood requirements during surgery and through the first 48 postoperative hours. Anesthesiologists and surgeons involved in the care of these patients believed that their clinical behavior during surgery and postoperatively was as good as that of patients subjected to similar procedures without preoperative phlebotomy. Also, their need of blood replacement was no greater than that of patients who underwent similar procedures without autologous transfusion.

Milles, and associates[17] also studied the electrocardiograms of autologous-transfusion patients to see whether signs of ischemia (as reflections of decreased oxygen-carrying capacity due to loss of hemoglobin) might serve as a modified Master test. But patients whose patterns before phlebotomy were normal had the same patterns afterward; the patients with evidence of previous heart disease (myocardial infarction, etc.) showed only nonspecific S-T wave changes after phlebotomy. In some cases where patients had electrocardiographic evidence of ventricular hypertrophy before phlebotomy, the pattern improved after phlebotomy. This probably was due to a reduction of cardiac work load by decrease of blood volume and viscosity.

Several investigators have reported removing 3 or more units preoperatively without problem.[18,19,23,24,29,53-55] Milles and associates[17] reported three 1-unit phlebotomies in several cases. Two of the patients were men, aged 32 and 42, who were to undergo lobectomy. Units were drawn 8, 6, and 4 days before surgery.* Hemoglobin values mea-

*AABB standards recommend a 4-day interval between phlebotomies and at least 72 hours between the last phlebotomy and surgery. This permits preoperative acquisition of 4 units of blood with conventional storage techniques.[14,15]

sured before the first phlebotomy and just before the operation were 15.8 and 13.2 g/dl in the first case and 15.0 and 11.0 in the second.

Sands and associates[54] reported similar results in patients undergoing radical surgery of the head and neck. The lowest preoperative hemoglobin concentration in their group was 11.6 g/dl, which occurred in a 61-year-old man who had undergone hemiglossectomy, partial mandibulectomy, and neck dissection after donating 3 units of blood in the 3 weeks preceding surgery. Before phlebotomy, the value had been 15.3 g/dl. In two other patients, the hemoglobin fell 2 g/dl (14.9 to 12.9) and 2.3 g/dl (14.4 to 12.1) after three 1-unit phlebotomies. None of these patients received iron supplement.

Other investigators also have reported using multiple phlebotomies. Cuello and associates[23,24] removed as many as 3 units before surgery from patients who were to undergo various thoracic and cardiovascular procedures. These patients were given iron intramuscularly. Newman and co-workers[19] performed three or more phlebotomies preoperatively on patients undergoing open-heart surgery for correction of acquired heart disease. Among 20 males the mean decrease of hemoglobin was 2.2 g/dl (15.5 to 13.3), and among 18 females the mean decrease was 3.2 g/dl (14.6 to 11.4). Each of these patients was given 250 mg of iron-dextran (Imferon) intravenously with each phlebotomy. Nine of the 178 patients who had one to four phlebotomies had adverse reactions to the iron. To avoid such reactions, Zuck and Bergin[29] administered iron orally to 18 patients undergoing multiple phlebotomies for autologous transfusion. Eight patients were bled 4 units, seven patients 5 units, two patients 6 units, and one patient 8 units. The mean decrease of hematocrit was 9.3% (range, 1.4 to 16%), none falling below 33%. The mean reticulocytosis reported was 5.5% (range, 1.5 to 15%).

In all patients mentioned above subjected to multiple preoperative phlebotomies, none came to operation with hemoglobin less than 10 g/dl. This level of hemoglobin has been regarded by many anesthesiologists as the minimum acceptable level before an elective surgical procedure.[56-59] There is evidence, however, that patients with acute and chronic anemias (for example, anemia of chronic renal disease) and less than 10 g/dl hemoglobin tolerate anesthesia and the postoperative period much the same as those with normal hemoglobin values.[58] In normal patients (hemoglobin 15 g/dl, cardiac output 5 liters/min, arterial oxygen saturation 98%), approximately 1,000 ml of oxygen per minute is available to the tissues; minimum levels tolerable appear to be 400 ml/min.[58,60] Thus, provided that patients have adequate mechanisms to compensate for their anemia, they should be able to tolerate hemoglobin levels of 10 g/dl and less. These compensatory mechanisms include rightward shift of the oxyhemoglobin dissociation curve, decrease of blood viscosity, decrease of peripheral resistance, and increase of cardiac output.[48] This information adds justification to the concept of preoperative autologous transfusion, since patients undergoing multiple preoperative phlebotomies maintain hemoglobin levels well above the level needed to provide minimum oxygen delivery to the tissues.

Investigators have shown that autologous transfusion is ideally suited for women of child-bearing age, children, and young adults, since isoimmunization during youth or in the child-bearing years can complicate future transfusion needs or pregnancies. Milles and associates[61] and Peddle and associates[9] reported use of preoperative autologous transfusion for women about to undergo cesarean section. Others have reported its use in children and young adults.[19,29] Cuello and associates[23,24] reported using this form of transfusion in a child as young as 4 years. Recently Cowell and Swickard[62] described an autologous transfusion program in 193 children undergoing various orthopedic procedures. These children ranged in age from 7 to 20 years (average age, 14), and 1 to 3 units were drawn from each before surgery. The amount of blood withdrawn at each donation was determined by the weight of the child (>48 kg, 450 ml; 43 to 48 kg, 400 ml; 36 to 42 kg, 325 ml; 30 to 35 kg, 250 ml; and <30 kg, 100 ml). The average preoperative decrease of hemoglobin in these children was 2.1 g/dl, giving them an average preoperative level of 11.5 ± 1.6.

Frozen Red Cells.—Frozen storage of blood (at −80°C or colder) greatly increases the applicability of preoperative autologous blood transfusion. More than 80% of the red cells from a unit of blood stored frozen for 10 years by current methods can be expected to survive and function normally after transfusion.[63,64] Reported advantages of frozen red cells include higher levels of diphosphoglycerate (2,3 DPG) and adenosine triphosphate (ATP) (similar to levels present on the day they were frozen); the possibility of salvaging outdated red cells by freezing after rejuvenation with pyruvate, inosine, guanosine, phosphate, adenine, and glucose, which restores levels of ATP and 2,3 DPG[65-67]; and the fact that frozen red cells initially intended for autologous use but not

needed can subsequently be used for homologous transfusion.

There are some drawbacks, however, in the use of frozen red cells for autologous transfusion. Currently the process of freezing red cells is more expensive than conventional storage at 1 to 6°C and is generally unavailable except in larger centers. In addition, reconstitution of each unit of frozen red cells takes approximately 30 minutes; current regulations (which may prove somewhat restrictive[68]) require that they be infused within 24 hours after thawing, since longer storage may permit bacterial growth. Consequently, frozen red cells often are not convenient for standby surgical use.

Applications of frozen red cells in autologous transfusion have been diverse. They have been used for elective gynecologic, gastric, and oral surgery.[69-71] Åkerblom and associates[72] have used frozen autologous red cells for transfusion during maintenance hemodialysis and during transplant surgery. Seven patients donated a total of 33 units despite anemia (hemoglobin concentration of 7 to 12 g/dl) and uremia (serum creatinine of 5 to 16 mg/dl). Units were donated at approximately 8-week intervals without difficulty. Although conflicting data exist,[73] it seems appropriate to use autologous blood in patients with renal disease, since most of the current evidence indicates that patients in whom cytotoxic antibodies develop secondary to homologous blood transfusion have a higher incidence of transplant rejection.[8,74] Frozen autologous red cells have also been used in orthopedic, gastric, and open-heart operations on patients with rare blood types[75-77] and antibodies to high-incidence antigens.

HEMODILUTION AND AUTOLOGOUS TRANSFUSION

Hemodilution and autologous transfusion are now being used by several surgeons and anesthesiologists in this country for open-heart surgery. It is basically an extension of the preoperative form of autologous transfusion, performed by infusion of a hemodiluent in one vein and simultaneous phlebotomy from another to produce a state of normovolemic anemia. This is done just prior to heparinization and before extracorporeal circulation. After surgery, the unit or units of blood previously phlebotomized are infused. Hemodiluents employed include both crystalloids (Ringer's) and colloids (salt-poor albumin, dextran, plasma protein fraction). Because the degree of hemodilution can become sizable, this method of autologous transfusion is particularly suited for patients undergoing extracorporeal circulation, since most are aided by extensive monitoring and many undergo protective hypothermia.

The rationale of this practice is that hemodilution causes a considerable reduction in the number of red cells lost during operative bleeding and thus a decrease in subsequent need of homologous blood. The volume of blood saved is directly proportional to the difference between the original and the postdilution hematocrit levels. Diminished use of homologous blood has been reported by several investigators along with a decrease in the incidence of hepatitis.[78,79] It was hoped that another benefit from this form of autologous transfusion would be a decrease of coagulation problems following surgery, since the autologous units are fresh and high in coagulation factors. But coagulation studies have not shown this to be true,[78,79] even though platelet counts have increased after transfusion of this fresh autologous blood.[79-81]

In support of this form of autologous transfusion, it has been shown in dogs that cardiovascular function can be maintained despite acute and severe normovolemic anemia induced by hemodilution with hematocrit as low as 5%.[82,83] Others have shown that acute normovolemic anemia (hematocrit 10 to 15%) does not significantly alter left-ventricular function or increase the severity of experimentally induced myocardial ischemia.[84,85] Blood flow is maintained to the myocardium, most likely by an increase in collateral flow secondary to a decrease in viscosity.[85] This has particular significance in patients with coronary artery disease. Others have shown that adequate oxygenation of the tissues occurs during hemodilution despite severe diminution of red-cell volume.[86-88] Pavek and Carey[89] demonstrated that in spite of decreased oxygen availability, uptake by the tissue increases enough to maintain oxygenation. Wise and associates[90] have shown that the compensatory responses to acute anemia induced by hemodilution are well maintained, at least until more than 50% of the total circulating hemoglobin is lost. Rush and Eiseman[91] observed a similar result in dogs hemodiluted to hematocrits of 16%.

If these data from dogs are applicable to humans, then moderate hemodilution should be tolerated by most patients. Rush and colleagues[92] have adopted a hematocrit level of 28% as the limit of allowable dilution in the replacement of blood by crystalloid solutions during hemorrhage. In patients undergoing total hip arthroplasty with appropriate anesthesia, Laks and associates[93] have found a hematocrit level of 21% to be safe. However, patients under-

going open-heart surgery can tolerate even more severe hemodilution. Buckley and associates,[94] in a study of 17 patients (aged 2 weeks to 28 years) undergoing hemodilution and autologous transfusion with correction of congenital heart defects, showed 10% hematocrit to be sufficient during extracorporeal circulation. Seager and associates[95] have reported successful use of hemodilution and exchange transfusion with hypothermia and extracorporeal circulation in treatment for an incompatible blood transfusion. During the procedure, the hematocrit was as low as 3%.

The cardiac anesthesia group at the Massachusetts General Hospital has been very active in this form of autologous transfusion and has used it more than 800 times with various cardiac procedures since December 1970. Recently they reported a series of 50 patients undergoing cardiac surgery, 25 of whom were subjected to hemodilution and autologous transfusion. In the period between induction of anesthesia and administration of heparin, approximately 2 units (1,000 ml) of blood were withdrawn from each of these 25 patients through an arterial cannula into plastic bags containing citric acid, trisodium citrate, and dextrose solution (ACD). After the phlebotomy, blood pressure and heart rate were maintained with Ringer's lactate and, when needed, homologous blood. The autologous blood was transfused at the end of perfusion, after administration of protamine. Overall, the 25 patients transfused with autologous blood needed an average of 860 ml less of homologous blood products, thus reducing the blood requirement by 25%. The mean hematocrit value in this group, after removal of autologous blood but before bypass, decreased 6.7 ± 3.4% versus 1.8 ± 2.0% for the control group. Coagulation studies showed no significant improvement in those patients who received fresh autologous blood.[78]

A group at the Ochsner Clinic[79] have reported on a series of 150 patients who underwent cardiac bypass procedures with hemodilution and autologous transfusion. The age of these patients ranged from 2 months to 77 years. For autologous transfusion, immediately before heparinization and prior to commencement of extracorporeal circulation, 20% of the blood volume of each patient was withdrawn and twice its volume of Ringer's lactate was infused to maintain cardiovascular stability. Patients of this group required an average of 1,622 ml less homologous blood than did those of the control group. The incidence of post-transfusion hepatitis was less in the group that received autologous blood and was related directly to the number of homologous units used. Hematocrit levels dropped to 30% after phlebotomy and to as low as 15% during bypass. The partial thromboplastin times, prothrombin times, and clotting times measured before phlebotomy and after autologous transfusion did not differ significantly; but platelet counts did rise an average of 28,000/mm^3 following the infusion of the fresh autologous blood.

The value of hemodilution and autologous transfusion lies in the fact that it significantly decreases the amount of homologous blood needed. Because of the severe hemodilution, care is necessary in employing this technique. It is best suited for patients undergoing extracorporeal circulation and protective hypothermia.

INTRAOPERATIVE AUTOLOGOUS TRANSFUSION

The concept of transfusing a patient's own blood retrieved during surgery to maintain circulatory stability is far from new. Blundell[96] probably was the first to apply it, in 1818; but the first article advocating intraoperative autologous transfusion was published by Highmore[97] in 1874. His use of intraoperative autologous transfusion was reported to have been successful for patients with massive hemorrhage in many different types of surgery. By 1960 the literature contained reports of its employment in several hundred cases of massive hemorrhage.[98-119] The history of intraoperative autologous transfusion has been reviewed by Wilson and Taswell.[120]

During the early years of intraoperative autologous transfusion, various techniques were employed to collect the blood for reinfusion. Many surgical teams merely scooped it from the wound with a cup or mopped it up with gauze swabs. The blood was then filtered, sometimes citrated, and diluted with saline before reinfusion.[109,121] Others collected blood from the thorax via chest tubes and reinfused it after filtration.[122-124] Still others employed suction devices.[109,125,126] In 1960, Weekes and associates[127] proposed a closed system for intraoperative autologous transfusion in which blood was sucked through a filter and collected in a bottle that was turned upside down when full for the reinfusion.

Before 1960, there was little mention of problems associated with intraoperative autologous blood transfusion. Stager[126] in 1951 noted hemolysis secondary to a suction technique, and Backer-Gröndahl[121] in Norway in 1953 noted hemoglobinuria that he attributed to hemolysis due to the mechanical trauma of intraoperative blood salvage. It was not until 1966, however, that some of the problems involved, such as hemolysis and microembolism,

were appreciated fully. It was Dyer[128] who at this time designed an apparatus that he believed responded to some of these problems. It consisted of a two-chambered Pyrex unit connected to a suction apparatus selected to minimize hemolysis. Anticoagulation was accomplished by systemic heparinization of the patient or chamber anticoagulation. Blood foaming was prevented by spraying the inner surface of the chamber and the filters with silicone. Three sets of filters were used to remove much of the debris collected along with the salvaged blood. Reinfusion of fat was prevented by designing the collecting chambers so that fat would float to the surface and the last 100 ml of blood, containing the fat, would not be reinfused.

In 1968 Klebanoff and Watkins[129] described a new apparatus. This was to become the first commercially available and the most widely used device for intraoperative autologous transfusion, and today a similar device is marketed as the Bentley Autotransfusion System (Bentley Laboratories, Santa Ana, CA). It consists of two components: a disposable section that includes a sterile reservoir, filter, tubing, and a suction device; and a steel cabinet containing a variable-speed roller pump, controls, and a warning device indicating when the level of blood in the reservoir is low and there is danger of air embolism.

Anticoagulation can be accomplished either by systemic heparinization of the patient or by instillation of heparin or citrate anticoagulants into the collection reservoir. Filtration is done by a nylon 125-μm mesh filter located in the reservoir and two standard in-line blood filters. The reservoir is primed with various saline solutions.

In the same year, 1968, Wilson and Taswell[120] proposed utilization of a continuous-flow centrifuge for intraoperative autologous transfusion. This device, unlike the Bentley, could wash red cells and eliminate much of the unwanted debris from the reinfused blood. It was employed initially in patients undergoing transurethral resection.[130,131] A similar continuous-flow centrifuge device was subsequently used to salvage blood during surgery by Watson-Williams and associates[132] and Gilcher and Orr.[133]

Recent Investigations.—Klebanoff and associates[134,135] were the first to report on clinical use of the Bentley system. In 100 cases, they salvaged and reinfused a total of 213,320 ml of blood. Most of these autologous transfusions accompanied emergency surgery for trauma, but some elective vascular and orthopedic operations were included. Of the 100 patients, 84 survived and 16 died. Complications included hemoglobinemia, post-traumatic pulmonary insufficiency, disseminated intravascular coagulation (DIC) syndrome, and air embolism.[136]

Brener and associates[137] reported on clinical use of Klebanoff's device in 20 cases of elective vascular surgery at the Massachusetts General Hospital. They substituted a Swank micropore filter for the standard blood filters. The amounts of blood salvaged ranged from 200 to 15,000 ml, and the saving of homologous banked blood was considerable.[137] The complications Brener and associates reported were similar to those of Klebanoff.

Reports of the use of the Bentley Autotransfusion System are numerous[138-145] and come mainly from large trauma centers, where the need for blood is frequently urgent and surgery cannot be delayed while the blood bank prepares homologous blood. Dowling[142] at Charity Hospital in New Orleans has used a total of 49,200 ml (98 units) of transfused autologous blood in 34 cases. In five patients who had sustained thoracic trauma, Bregman and associates[145] salvaged approximately 7,200 ml of blood per patient with the Bentley device. Rakower[143] at Bellevue Hospital in New York used 30,000 ml of autologous blood in one patient with a stab wound that had lacerated both atria and the pulmonary vein. Complications reported by these authors were similar to those reported by Klebanoff.

Success with the use of the continuous-flow centrifuge device for autologous transfusion also has been reported. Wilson and co-workers[130] used this device in 11 instances of transurethral resection. Blood and irrigating fluid were collected from the surgical resectoscope and passed into a centrifuge. The cellular components were washed and the plasma and wash fluid, with their contaminants, were discarded. This method is similar to that used for washing and deglycerolization of frozen red cells.[146] After washing, the cellular components were resuspended to a desired hematocrit value in Ringer's lactate solution for return to the patient's circulation.

This type of autologous transfusion process appears to be superior to the Bentley system, since the red cells are washed free of contaminants, which are discarded with plasma and wash fluid. With this system and the introduction of wash fluids of selected density gradients, it may be possible to remove most of the contaminants in present-day intraoperative autologous transfusions.[147] These contaminants include free hemoglobin, microemboli, and red-cell phospholipids implicated in DIC.[148] In contrast to experience with the Bentley Autotransfusion System, Wilson and associates[130] found no evidence of elevated plasma hemoglobin in their 11

cases. This was corroborated in another study, by Kingsley and associates,[149] in which baboon autologous blood was washed free of plasma hemoglobin with a continuous-flow centrifuge.

Complications.—There are a number of problems associated with intraoperative autologous transfusion which have limited its acceptance. Complications reported by investigators include:

1. Hemolysis
2. Coagulation disorders
3. Microembolism
   a. Fat
   b. Denatured protein
   c. Microaggregates (platelets, leukocytes)
4. Air embolism
5. Sepsis and metastasis if used in patients with infection or malignancy.

Hemolysis.—Several authors[136,140,141] have shown a significant increase in plasma hemoglobin levels after autologous transfusion with the Bentley system, usually with a return to normal in 48 to 72 hours after surgery.[137-141,144] Brener and associates[137] reported a mean postoperative plasma hemoglobin level of 175 mg/dl with a high level of 600. Before reinfusion, Brener and associates[137] and Aaron and associates[150] found free hemoglobin levels in the salvaged blood as high as 1,600 mg/dl (normal in most laboratories is less than 20). Red-cell destruction is a result of trauma from the vacuum, compression effects of the roller pumps, contact of blood with air and tissue, and turbulent flow and foaming in tubing and reservoirs.[151,152]

High levels of free hemoglobin generated by the autologous transfusion process may be nephrotoxic. Renal damage has been associated with elevated free hemoglobin, but controversy surrounds the cause. It is uncertain whether the free hemoglobin molecule itself, the more toxic breakdown products of hemoglobin, or the lipid fraction of the red-cell stroma causes the nephrotoxicity associated with hemolysis.[153-159] An accelerated intravascular coagulation mechanism may be responsible for the hemolytic nephrotoxicity.[160-162] Reinfusion of hemolyzed autologous red cells is known to be capable of inducing intravascular coagulation in the dog.[163] Whatever the cause, however, all authors employing intraoperative autologous transfusion have reported that with adequate hydration they apparently can prevent this complication. With only a handful of studies on this problem, and with uncertainty as to its cause, hemolytic nephrotoxicity still remains a potential problem.

With intraoperative autologous transfusion, the hematocrit value decreases and usually remains depressed for several days. Brener and associates[137] have shown a decrease of 10% following use of the Bentley system; and Wilson and co-workers[130] observed a decrease of approximately 4% with the continuous-flow centrifuge. Lessening of hematocrit is common after most surgical procedures, but the decline after intraoperative autologous transfusion has a somewhat different mechanism. The decrease of hematocrit is due to hemolysis, loss of red cells in the system and filters, and dilution by the saline priming solutions. It is not likely that the transfused red cells are destroyed rapidly in vivo, as suggested by Mati and associates.[164] They studied erythrocyte survival in blood obtained from the peritoneal cavity of patients transfused with autologous blood salvaged during ectopic pregnancy. Using ^{51}Cr-tagged red cells, they determined that the half-life of erythrocytes collected from the peritoneal cavity was approximately 8 days while that of normal venous erythrocytes was approximately 60 days. This differs from observations by Wilson and co-workers[130] who employed the continuous-flow technique in humans, by Bennett and associates,[165] who used the Bentley system in dogs, and by Symbas and associates,[122] who reinfused blood obtained from a hemothorax. Each of these three studies showed that transfused autologous erythrocytes and control erythrocytes did not differ significantly in survival time.

Although there is considerable damage to the red cells during intraoperative autologous transfusion, Dyer and associates[166] have suggested a potential advantage in the transfusion of those red cells that do survive. Since these cells are fresh, their 2,3 DPG level is presumably normal. Stored red cells are known to be depleted of 2,3 DPG; and because of this, the oxyhemoglobin dissociation curve shifts to the left: the result is lowered oxygen tensions at which hemoglobin releases oxygen.[167,168] Research concerning the oxygen transport of red cells salvaged for autologous transfusion is lacking. Aaron and associates[150] have demonstrated a higher oxygen tension in autologous blood than in banked blood. In addition, Carty and Barr[169] have determined that the hemoglobin obtained from the intraperitoneal cavity for autologous transfusion is predominantly intracellular and thus has the potential for normal oxygen transport.

Coagulation Disorders.—Another major area of difficulty is the apparent detrimental influence of intraoperative autologous transfusion on coagulation.

During transfusion, interfaces of blood with air and tissue can cause significant platelet aggregation and subsequent thrombocytopenia. In addition, activation of the coagulation mechanism can occur and lead to intravascular coagulation.

A. Thrombocytopenia.—Severe thrombocytopenia often follows any form of extracorporeal bypass such as intraoperative autologous transfusion. In has occurred after exposure of blood to pleural surfaces and to peritoneal surfaces.[124,137,139,144,150] Both Bennett and associates[165] and Rakower and associates[170] have reported that the decrease of circulating platelets is greater in blood exposed to tissue cavities than in blood salvaged without extravascular tissue contact. This thrombocytopenia can be aggravated by addition of micropore filters to the system, since fibrin clot formation on the filters traps and removes platelets from the system.[140,171,172]

The degree to which this thrombocytopenia is deleterious to the patient is unknown, although it is generally agreed that platelet counts greater than 50,000 to 65,000/mm^3 prevent hemorrhage during surgery.[173,174] For platelet counts below this level after autologous transfusion, Rakower[143] has given platelet concentrates. In addition to the thrombocytopenia, Brener and associates[175] have shown that platelet metabolism and aggregation are significantly altered during intraoperative autologous transfusion. However, they maintain that platelet function returns to normal in the patient after reinfusion of the damaged platelets.

B. Intravascular Coagulation.—Development of hypofibrinogenemia and fibrin split products and prolongation of the thrombin, prothrombin and partial thromboplastin times have been frequent during intraoperative salvage and reinfusion.[124,138,139,141,144,145,170] In fact, Duncan and associates[144] have reported the occurrence in three subjects of severe coagulopathy with resultant death throught to be related directly to use of the Bentley device. Rakower and associates[170] have reported that a constant finding after their use of intraoperative autologous transfusion was a generalized oozing of blood from all cut surfaces; this increased the operating time by as much as 3 hours before hemostasis was achieved. Heparin reversal and administration of coagulation factors with fresh-frozen plasma infusions had little effect in stopping this bleeding. As Rakower and associates pointed out, these findings are consistent with DIC syndrome induced by the autologous transfusion process. Others also have reported the development of DIC with intraoperative autologous transfusion.[135,136,138,140,141] However, most of these instances have occurred in patients who had considerable tissue damage and who also had received large amounts of both autologous and homologous blood. Beller and associates[176] and Carty and associates[177] have shown that peritoneal blood with ruptured ectopic pregnancies (blood that may be salvaged and reinfused) has pronounced depletion of coagulation factors and a grossly elevated level of fibrin degradation products. The autologous transfusion process may be the primary cause of the DIC or it may merely aggravate an already existing coagulopathy.

Many patients undergoing extensive surgical procedures have an element of intravascular coagulation induced by both endothelial-cell injury, which activates Factor XII and the intrinsic clotting mechanism, and tissue injury, which activates the extrinsic clotting system. In addition, it is known that both red-cell destruction and platelet aggregation occur on a large scale during intraoperative autologous transfusion and that products of red-cell and platelet injury, such as coagulant phospholipids, are powerful thromboplastic substances capable of initiating intravascular coagulation.[148,178] Thus, massive autologous transfusion can induce intravascular coagulation or contribute to an already existing process leading to a perpetuating cycle of increased bleeding.

Some have recommended that, as a safeguard against this, one should limit autologous transfusion to the period of major blood loss in order to minimize the deleterious effect of the process on the clotting mechanism.[179] Evidence currently available suggests that autologous transfusion done on a smaller scale with the Bentley device (reinfusion of less than 3,000 ml) does not disrupt the coagulation mechanism to any major extent and can be employed safely in these instances.[137-139] Others have advocated—and demonstrated in the baboon—that DIC can largely be prevented by washing the autologous blood with a continuous-flow centrifuge that removes thromboplastic substances and prevents activation of the coagulation mechanism.[149] With a washing technique, any resultant coagulopathies would be dilutional in nature and could be readily corrected with appropriate component therapy.

Microemboli.—Evidence for the presence of microemboli or microparticles (>20 μm in diameter) in autologous blood lies in the elevation of screen filtration pressures by this blood. Results of particle-size analysis also indicate microparticles produced mainly by platelet aggregation.[137,150,180-182] Microembolization of platelet aggregates and other debris has been implicated in the development of pulmo-

nary insufficiency and neurologic complications following trauma, massive transfusions, shock, and extracorporeal circulation.[178,183-188] The extent to which microembolization occurs after intraoperative autologous transfusion is unknown, but pulmonary insufficiency has been reported by Dowling,[142] Klebanoff,[136] and others.[138,139]

Emboli that occur during autologous transfusion usually consist of one or a combination of the following:

Fat, fibrin, and foreign material removed from the wound, missed by the filters, and reinfused to the patient.
Denatured plasma lipoproteins that release chylomicrons, which form embolic globules.[189]
Platelet and leukocyte microaggregates and red-cell membranes, which result in elevation of screen filtration pressures.[190]
Air embolus.

To study the efficacy of reinfusing autologous blood contaminated with bone fragments and marrow, Dorang and associates[191] performed autologous transfusion in conjunction with spinal fusion in 10 dogs, using the Bentley Autotransfusion System. After surgery, the animals were killed and gross and microscopic examination of the organs was undertaken. No bone or cartilage was found in any of the tissues studied, and evidence of fat embolization was seen in only one dog. Further studies with appropriate controls are needed to confirm the safety of autologous transfusion in surgical procedures in which there is a risk of bone and fat emboli.

Denaturation of lipoprotein leads to a release of chylomicrons, which aggregate and form embolic globules.[192] Experiments in dogs revealed the presence of such microemboli in the lungs, kidney, and brain after autologous transfusion. Results indicated that protein molecules that bind or transport lipids can break down when exposed to an air-blood interface or when exposed to negative pressure. Both conditions can be present in an autologous transfusion system.

In addition to these forms of emboli, platelet and leukocyte microaggregates may be present during autologous transfusion—especially in blood retrieved from body cavities, where platelets come into contact with connective tissue and undergo aggregation.[165,170] The effect of these microaggregates in the subsequent development of systemic microemboli probably is similar to their effect following massive homologous blood transfusions.[186,187] Use of micropore filters during autologous transfusion, as in homologous transfusion, removes many of these microparticles.[193,194] Brener and associates[137] and Bennett and associates[165] have shown that autologous blood passes through micropore filters with less screen filtration pressure than does homologous blood; and Wright and Solis[182] found a decrease in the volume of microparticles remaining in autologous blood after ultrafiltration. Although this is still controversial, some authors[171,195] maintain that these filters reduce pulmonary morbidity and mortality in humans after massive infusions of banked homologous blood. If this is so, filters may also be of benefit in autologous transfusions. However, they do not remove the serotonin, histamine, and catecholamines liberated by platelet aggregation. These potent vasoconstrictors can cause bronchoconstriction, increased pulmonary vascular resistance, and pulmonary hypertension, and thus create pulmonary insufficiency in spite of the use of micropore filters.[172,196,197]

Recently, some investigators have concluded that fresh autologous blood intraoperatively transfused, is less injurious to the lung than is banked autologous blood. Bennett and colleagues[198] studied the pulmonary effects in dogs of both autologous blood salvaged from the pleural cavity and banked autologous blood. In their experiments, transfusion of 3-week-old banked autologous blood induced greater increases of pulmonary vascular resistance and bronchial pressures and greater decreases of arterial-venous oxygen partial pressure differences than did transfusion of fresh autologous blood. Postmortem examinations revealed significantly greater evidence of interstitial pulmonary edema, perivascular hemorrhage, intra-alveolar fluid, and alveolar congestion in the dogs that had received the 3-week-old banked blood. Also, Rakower and associates[170] were unable to find evidence of multiple pulmonary emboli by postmortem examination of dogs transfused with fresh autologous blood. Wright and associates[199] reported that autologous transfusion to the subhuman primate of up to four times its blood volume can be done without significant development of microaggregates or pulmonary dysfunction, provided the animal has been adequately heparinized and kept normotensive. They suggested that hypotension and trauma, rather than the autologous transfusion per se, render patients susceptible to platelet aggregation. Their observations imply that the storage lesion of blood may be associated with the development of posttraumatic pulmonary insufficiency and that patients who undergo intraoperative autologous transfusion without

significant problems such as shock and trauma may have fewer postoperative pulmonary problems.

Air embolism, when it occurs, is usually due to faulty technique in using the Bentley system. Air embolization occurs when those operating the machine fail to note that the blood level in the reservoir has fallen below the safe level (200 ml). Air then makes its way into the blood lines and into the patient. To help prevent this complication, a photoelectric alarm has been installed in the Bentley system to warn against low blood levels in the reservoir. But, as Reul and associates[138] point out, this alarm cannot be relied on since clots within the reservoir may obscure and subsequently silence the alarm. Fatalities due to air embolization with the Bentley system have been reported by both Klebanoff[136] and Mattox and associates.[139]

Current Limitations.—Intraoperative autologous transfusion is generally not employed in cases of infection or malignancy, lest widespread septicemia or metastasis result. Yaw and associates[200] have demonstrated malignant cells in blood salvaged with the Bentley system. However, Klebanoff and associates[201] found no incidence of sepsis and a negative blood culture 24 hours after contaminating a wound in dogs with fat, bile and feces and reinfusing blood collected from the wound. Wilson and associates[130] found that no complication resulted from inadvertent transfusion of washed autologous blood to a patient who had undergone transurethral resection and whose preoperative urine cultures were positive. Sepsis did not develop; and the postoperative rise of temperature was minimal, as it was in 11 of 15 other patients studied who underwent transurethral resection without autologous transfusion. Well-controlled studies as well as refinements in technique are needed before autologous transfusion can be deemed acceptable in patients with malignancy or infection.

There remains much disagreement about the best method for anticoagulation, and investigators stress that proper anticoagulation is necessary to limit complications.[138,139,144,145] Some investigators advocate reservoir anticoagulation with heparin or ACD; others favor systemic heparinization.[136,141,202,203] Patients undergoing routine elective surgery with autologous transfusion probably can tolerate systemic heparinization; but in those cases of major trauma with associated injuries, as in head trauma or closed extremity fractures, systemic heparinization may well be contraindicated.

There are many problems to be solved before intraoperative autologous transfusion becomes widely accepted. The advent of the Bentley system has generated a great deal of interest, but clinical investigative studies have uncovered enough problems to question its general acceptance. In addition, many of these studies have been without appropriate controls and many have touched only lightly upon the problems of hemolysis, disruption of the coagulation mechanism, and microembolization. New methods of intraoperative autologous transfusion must be developed which not only will solve these problems but also will offer hope that autologous transfusion can be used for patients who cannot undergo systemic anticoagulation, for patients with generalized infection, and for patients with malignant disease. Cell washing with the aid of continuous-flow centrifugation may provide an answer to these difficulties. The Haemonetics Corporation has now developed a prototype device for autologous transfusion which incorporates into one machine (Cell Saver, Haemonetics Corporation, Natick, MA) a reservoir, filter, and means of cell washing.

COMMENT AND CONCLUSION

The development of autologous transfusion as a routine procedure has been long neglected. It is now widely recognized that the demand for blood will continue to exceed available reserves in many areas of the country. Advances in surgical technology and a rapidly increasing volume of surgery contribute to the increasing demand for blood. Recent legislation in some states and the forthcoming National Blood Policy discouraging use of paid donors have put an added strain on blood reserves. In addition, the continued waste of blood due to outdating contributes to the blood shortage.

Transfusion of homologous blood sometimes causes significant complications in the recipient. The most frequent are febrile and allergic reactions. Isoimmunization to red-cell and HL-A antigens increases the possibility of future hemolytic reaction and possible graft rejection.[204] Circulatory overload and transmission of disease can be serious: it has been reported that from 2.8 to 16% of multiply transfused patients experience symptomatic post-transfusion hepatitis.[205,206] Reported mortality rates from hepatitis range from 0.1% to as high as 20% in older age groups.[205-207]

The problems and complications associated with collection and administration of homologous blood indicate the need for safer and more efficient methods of transfusion. It is reasonable to predict that with further development, autologous transfusion, in all three forms discussed, will prove to be safer than

homologous transfusion. We advocate continued use of autologous transfusion and research with appropriately controlled studies to perfect this potentially superior method of blood transfusion.

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HISTORICAL VIGNETTE

The Hospital and the Surgeon's Reputation

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Rightly, it has been said, "A surgeon without a hospital is like a gardener without a garden."[1] The hospital is not only a necessity for the practice of modern surgery, it is also a rendezvous of one generation of surgeons with the next, where the ideals and skills of each may be transmitted to the other. A surgeon's career may come to a bloom or blight according to the hospital in which he works.

Few hospitals have a more interesting surgical past than the Royal Infirmary of Edinburgh. It was built in the 18th century as a community effort (Fig. 1). The owners of stone quarries gave stone and lime; merchants gave timber. Farmers furnished free transport of the materials. Carpenters and masons gave their labor. Even the journeymen masons helped. Since the hospital was intended to serve the sick poor, day laborers worked one day a month without pay.

The foundation stone was laid in August 1738, and in 1741 a 200-bed hospital was ready for patients. Located in the central part of the building under the dome was a steep, well-like, somber operating theater capable of holding 200 students. In these dark surroundings much brilliant surgery was to be performed.[2]

One of the first surgeons to operate and teach here was Alexander Wood (1725-1807), "Lang Sandy Wood," an amiable, convivial eccentric who was popular in the city because he never neglected his poor patients (Fig. 2). His dexterity and skill raised the reputation of the surgical department of the Royal Infirmary.[2] In 1779, Wood had the talented young John Bell (1763-1820) as one of his new students. Wood and Bell had a happy association, and Bell dedicated the first volume of his *Anatomy of the Human Body* to his teacher.

Bell made many contributions to surgery, especially in surgery of the blood vessels. He was one of the finest, most enthusiastic teachers of his day. Through his lectures and writings, which emphasized the teleologic significance of structures, Bell created the concepts of surgical anatomy. Unfortunately, along with his brilliance he had a most persistent contentiousness and a great pride in the correctness of his own opinion. The enmity and jealousy of the medical faculty was aroused both by Bell's devastating criticism and by the great success of his private anatomy lectures.

According to a 1785 agreement with the managers, the surgical patients of the Royal Infirmary were taken care of by all the members of the Royal College of Surgeons on a bimonthly rotation. This was most satisfactory to the surgeon, but sometimes the care of the patient was interrupted. In 1799, the learned and vitriolic James Gregory (1753-1821), a member of the governing board of the hospital, wrote a memorandum condemning the practice of rotation and advocating that six surgeons be given permanent appointments to the Infirmary.

This led to a frightful dispute, with excessively rude remarks being made by both sides. Bell, champion of lost causes, leading surgeon and teacher of anatomy, spoke for the junior surgeons. But the victory in the Infirmary went to Gregory's forces and Bell was barred from operating in the Infirmary. He never recovered from this disappointment.[3]

A critical but vivid description of the Infirmary in 1800 written by a military surgeon, Robert Jack-
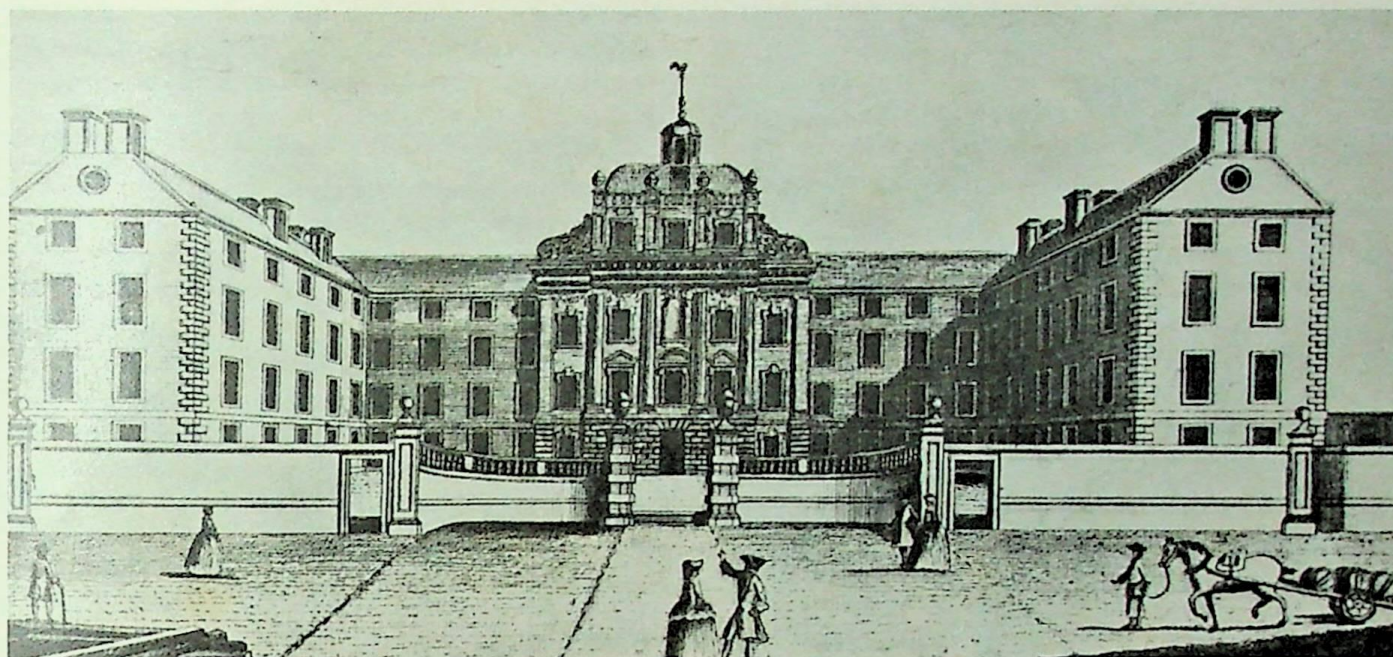
Fig. 1. Royal Infirmary of Edinburgh received its charter in 1736. Architect's plans, which are still extant, have legend, "This hospital will be open to all the cureable distressed from whatever corner of the world they come without restriction."[2]

son (1759-1827), revealed that the hospital, bound on one side by a high wall and on another by lofty houses, had small and poorly constructed windows which did not admit much air. The narrow, low wards were partitioned into many awkward cubicles. The grates were small and the fireplaces were so fenced in by the partitions that there was little circulation of air or heat. The frames of the bedsteads were made of iron, but the bottom part of the bed was formed of thick and spongy rope, full of dust and dirt. The straw-filled underbed, the flock mattress, and the bolster or pillow were usually soiled offensively. The sheets were dirty or ill-washed and the blankets and coverlets small and beggarly. Altogether, the appearance of the bed was unpleasant. The patients were allowed to keep their own clothing, even if it was dirty and ragged.[4]

In addition to the wretched physical plant, there were other reasons why the 18th century hospital was a grim place for both patient and surgeon. In the eloquent and compassionate "Memoir on the Surgical Diseases of the Poor" (1801), John Bell sensitively portrayed the inadequacies of even the best of the hospitals for the sick poor.

The sick individual so fears the hospital, he wrote, where he will have to face "the shame, the unhappiness, the pain of a public operation, the agony of being exposed before numbers of spectators," that he

> . . . lingers on in doubt and fear, till, at last . . . bereft by dreadful sufferings of every domestic comfort, become a burthen on his friends, he is at last conveyed to a Hospital, when too late to receive relief, his case only becoming an object of importance as a recorded instance and fatal warning of the incurable stage of his malady, or an example to students, of a desperate and unavailing operation. . . .[5]

The patient also knows, Bell continued, that "the infirmary is not simple charity—but reputed to be a school of experiments, and not much famed for successful operations." Bell gave his services to poor patients—one day a week until his poor health forced him to quit practice in 1817.[5]

Fortunately, Bell's methods of teaching were continued by his student, the popular John Barclay (1741-1823).[6] He collected many brilliant students and demonstrators, among them Robert Liston (1794-1847) and James Syme (1799-1870). In 1818, when Liston had a disagreement with Barclay, Syme became a demonstrator at Liston's own theater. But this collaboration between the distant cousins lasted only 5 years. Syme found work in the hospital more congenial than work in the dissecting room and he disapproved of Liston's methods of procuring material to dissect.

Because of this quarrel with Liston, Syme did not secure a needed academic appointment in 1829 and found the Royal Infirmary closed to him. But this did not defeat the great little man called the "Napoleon of surgery." He opened his own private 24-bed hospital at Minto House (Fig. 3), with an operating theater, lecture room, and accommoda-
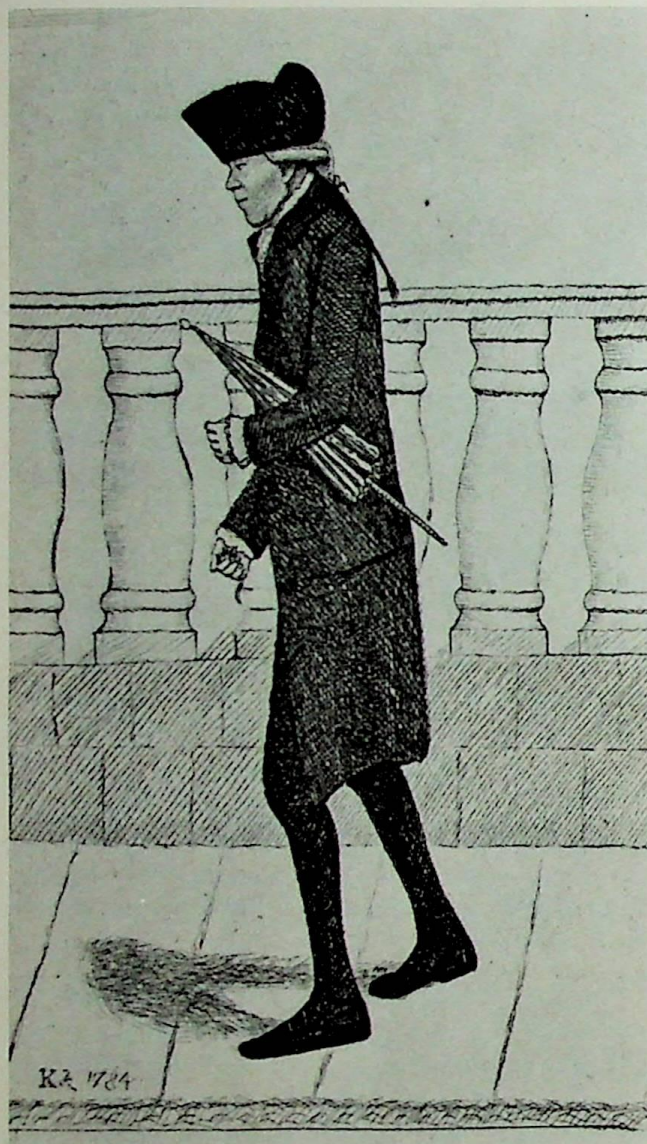
Fig. 2. Alexander Wood, "Lang Sandy Wood," was often seen walking along the streets of the city accompanied by his pet sheep and raven. As this illustration from Kay's *Portraits* shows, he carried an umbrella. He was one of the first in Edinburgh to do so.

tions for the resident staff of two house surgeons and several apprentices. The hospital was ready for patients on May 8, 1829. During the first 3 months 380 people asked for care, 70 were admitted into the house, and 30 operations were performed, with 2 deaths. Syme worked at Minto House for 5 years, performing and perfecting many innovative surgical procedures, particularly excisions of the elbow and knee joints and the first of his wonderful series of operations for aneurysm.[7]

One of the most famous of the operations Syme performed at Minto House was the removal of a cancerous breast. The patient was Ailie Noble, heroine of *Rab and His Friends,* and her story was told by Syme's assistant, John Brown (1810-1882). The patient, dressed in her ordinary clothes, accompanied by her husband and his dog, walked into the crowded and noisy operating theater.

> Ailie stepped up on a seat, and laid herself on the table, as her friend the surgeon, told her; arranged herself, gave a rapid look at James, shut her eyes, rested herself on me, and took my hand. The operation was at once begun; it was necessarily slow; and chloroform—one of God's best gifts to his suffering children—was then unknown. The surgeon did his work. The pale face showed its pain, but was still and silent.
>
> It is over; she is dressed, steps gently and decently down from the table, looks for James; then turning to the surgeon and students, she curtsies—and in a low, clear voice, begs their pardon if she behaved ill. The students—all of us—wept like children; the surgeon happed her up carefully—and resting on James and me, Ailie went to her room, Rab following.

Ailie died on the fourth day following the operation.[8]

Shortly after this event, Minto House ceased to be a hospital. In 1833, Syme was appointed Clinical Professor of Surgery to the University of Edinburgh, and the dark theater at the Royal Infirmary was then his domain. Under his leadership, surgery at Edinburgh regained its wonted luster.

Among Syme's students, in 1858, was a young Canadian, Donald Maclean (1834-1903). In later years, he became one of the most convincing spokesmen for Syme's doctrines in the United States, recalling admiringly Syme's skill as a surgeon and teacher, his role in adoption of sound principles in surgery, and his application to the theory and practice of surgery the best discoveries and deductions of abstract science. He wrote that Syme tried to deduce from his surgical experiences rules of practice that could be formulated into great gen-

Fig. 3. Minto House was a square, well appointed house of 15 rooms, located near the Infirmary in the densely populated part of the old city.
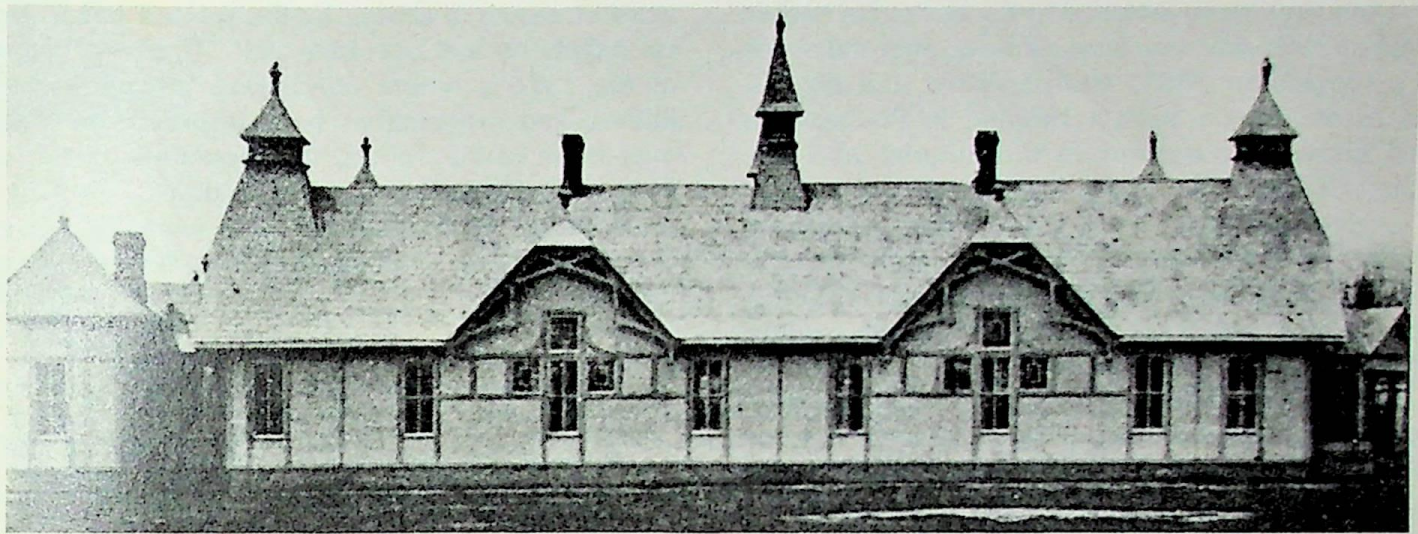
Fig. 4. Wooden pavilion hospital at the University of Michigan was designed by Edward S. Dunster, who had been military surgeon in the Civil War, and it was patterned after a military hospital. It could accommodate about 150 patients.[11]

eral principles for the future guidance and inspiration of surgeons.[9]

Maclean was given his great opportunity to influence the course of surgery when he was made Professor of Surgery at the University of Michigan, Ann Arbor, in 1872. The University of Michigan was a very progressive school for the American midwest, but it lacked an adequate teaching hospital. In 1875, the legislature made an appropriation of $5,500 for a hospital building and $2,500 for its equipment, provided that the citizens of Ann Arbor would raise $4,000 for the project. The citizens of the town responded quickly and generously, and the hospital was finished in 1877.

The building was of the pavilion type used in the latter part of the Civil War (Fig. 4). When built, it was understood that this hospital would become so badly infected within 10 years that it would be necessary to burn it. However, the building served as a hospital until 1890 and was then used for a class and laboratory building for 20 years more. It was a very good investment for the townspeople of Ann Arbor and the taxpayers of Michigan. Patients too poor to pay for medical or surgical treatment were admitted to the hospital and received free treatment from the professors of the medical school or from the hospital staff under their direction. The patients were charged $4.00 a week for board and hospital accommodations. They were also charged for medicines, dressings, or other appliances needed in surgical treatment.[10]

In these comparatively humble surroundings, Donald Maclean became one of the most fascinating and glamorous of Michigan's professors, greatly admired by the students. Handsome, bold, and dextrous, he was the beau ideal of the young men on the benches.[11] Maclean held his weekly surgical clinics in the morning, in the low-ceilinged amphitheater of the hospital, which was reached by outside stairs. Only seniors were required to attend, and the front seats were officially reserved for them. The juniors enforced their unofficial right to those next highest, and any freshman who cared to attend was relegated to the top row.

A surgeon, who was a freshman in 1880, later recalled,

> . . . sitting on the back seats, too far away to see the technic of the operation, but inspired by the fact that operations were going on; by seeing the assistants as they performed their duties; by seeing the members of the senior class called down to be quizzed on diagnosis and permitted to take some minor part in the operations.

His father had sent him to Michigan for just such experience, and he was determined to get it.[12]

And when this student was a senior, he had advanced to the position of Maclean's assistant. Later, in a discussion of a paper by his former assistant, Maclean said, "I have watched with the intense and critical observation of a father watching his son, the work of my former pupil Dr. Mayo, and I admire it very much indeed."[13]

One of the most important concepts that W. J. Mayo acquired at Michigan was that "the sick man was the hub around which the entire education turned; the application of the art of medicine was based on the science of medicine."[14]

When Will Mayo returned to Rochester, he had no hospital. The dramatic story of how, after the shock of a tornado in 1883, Mother Alfred and the Sisters of St. Francis built a hospital in Rochester is well known. It opened in the autumn of 1889. It had 50 beds, 4 nursing sisters, 1 trained nurse, a surgical assistant, Sister Joseph, and a superior, Mother Alfred.[15] Here W. J. Mayo began his magnificant career in which he made major contributions in the surgery of the stomach, colon, rectum, biliary passages, spleen, and kidney.

The surgical inheritance of W. J. Mayo included intellectual bequests from many great surgeons. His special genius and vision were his own, but the example of great surgeons such as Bell, Syme, and Maclean could have furnished helpful inspiration for his own great accomplishments. Enthusiasm for teaching, brilliance in improvisation, courage and excellence in execution, and consideration for the patient in opposing needless surgery could have been Bell's legacy. Syme bequeathed some of these qualities as well, and added counsel:

> Any one who practices physics or surgery as a profession should endeavour to render the means of relief which he possesses, as far as possible, available to those who require them, and with this view, place the liberality of the rich to the credit of the poor.[7]

Maclean set an example with his imposing presence and dedication to the highest ideals of his profession.

According to W. J. Mayo, "The reputation of a surgeon . . . must rest upon originality, teaching by word of mouth, teaching by the printed page, surgical judgment, and operative skill."[16] In the hospital of the 1970's, where conditions, attitudes, possibilities, and expectations have improved so greatly over those of the 1800's, the reputation of the surgeon still depends on these qualities. And he is remembered in the thoughts and works of his students, according to the degree in which he excels in them.

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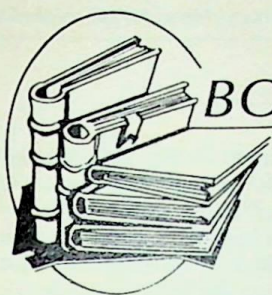
Cognitive Therapy and the Emotional Disorders, by Aaron T. Beck, 364 pp, with illus, $15, New York: International Universities Press, 1976

Cognitive therapy, as defined by the author, is a kind of common sense psychotherapy based on the theory that the emotionally disturbed individual, through introspective observation of his conscious thoughts and feelings, can be guided toward therapeutic awareness of the remedies for his psychologic conflicts. In contrast to the position posited by psychoanalytic theory, psychologic problems are not necessarily the product of mysterious unconscious forces but may result from commonplace processes such as learning incorrectly, making incorrect inferences, and not distinguishing adequately between imagination and reality. Central to the process of cognitive therapy is the idea of the automatic thought that is present but not reported during free association and is a separate train of thought running parallel to the reported thought content. Thus the cognitive therapist encourages the patient to apply the same problem-solving techniques he has used throughout his life to correct his fallacious thinking, using such cognitive processes as introspection, insight, reality testing, and learning. Essentially, this approach consists of greater interest in, and willingness to accept at face value, conscious thoughts, goals, and attitudes. The therapist focuses on the individual's conscious ideas.

The subject material is presented in an organized and eminently readable manner that has the manifest double purpose of describing cognitive therapy and its application and, secondly, convincing the reader of the scientific validity and even superiority of this type of psychotherapy. The author succeeds admirably in his first purpose, discussing the cognitive content of various emotional disorders—particularly neurotic anxiety and depression—and using case vignettes frequently to illustrate theoretic concepts. The strategy and techniques of cognitive therapy are discussed in a lucid style often lacking in other expositions on the proper conduct of psychotherapy.

Whether the author accomplishes his second purpose must be left to the judgment of the individual reader, who will be biased by his particular psychotherapeutic, behavioristic, or biologic orientation about the causes and remedies of emotional disorders. However, the open-minded eclectic psychotherapist should find the ideas presented intriguing and stimulating. At best, cognitive therapy may hold the promise (heretofore often unfulfilled) of allowing both the therapist and the patient to understand rationally what is happening in the mysterious if not mystic psychotherapeutic relationship. The book is recommended to both the aspiring and the experienced psychotherapist.

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The Patchwork Mouse, by Joseph Hixson, 240 pp, $7.95, Garden City, NY: Anchor Press/Doubleday, 1976

In March 1974, while working at the Sloan-Kettering Institute for Cancer Research, Dr. William T. Summerlin admitted that he had inked black patches on white laboratory mice to support his claims of having made a transplantation breakthrough. Over the preceding 2 years he had described a new way to successfully transplant skin, corneas, and possibly entire organs from one animal to another by first incubating them in tissue culture. His mentor Dr. Robert A. Good, director of the institute, frequently had told other scientists and reporters that, if confirmed, Summerlin's work was a major immunologic advance which also had important implications in cancer. Cancer research dollars had supported Summerlin's work. When uncovered, Summerlin said that most of his erroneous claims had been based on the misunderstanding of experimental results. He also blamed his actions on extreme personal pressures as a result of very heavy clinical and research responsibilities and cited Good for placing him under great pressure to produce "progress" in research.

Was this episode of deliberate falsification of experimental results unique to the scientists involved? Or was there some truth to his excuses even though they didn't exonerate his actions? Although Summerlin apparently was not interviewed at any length by the author, leaving certain questions unanswered, I think that a strong effort was made to deal objectively with information at hand.

A major portion of the book was spent exploring the question of whether the climate of cancer research today, with its high rewards and strong temptations, was somehow at fault. Hixson broadly reviews the development of federal funding of cancer research, the politics involved, and the problems that can arise when large amounts of federal money are allocated for targeted research without full consideration of the capacity of current facilities to make use of the money effectively. Perhaps as a manifestation of the popular belief, current in America, that problems (a disease in this instance) can be conquered by passing legislation against them, Congress and the public may expect more than can be delivered. When scientists yield to temptations to promise more than can be accomplished, public disappointment in science is likely to ensue. The solution to the cancer problem will require the development of new fundamental knowledge; however, its acquisition cannot be placed on a timetable similar to those previously successful in applied research. I think that science writers can have an important role in helping prevent or reduce such communication gaps between science and the public by tempering enthusiasm in reporting with reality. Hixson agrees. He places responsibility on science writers for a larger accuracy than merely being sure a quotation is correct. He wants them to be able to evaluate and add perspective. I wish all reporters felt this responsibility.

Hixson uses the Summerlin case as an entry to the whole arena of cancer research. The relationship of some of the discussion to the original patchwork mouse is rather tenuous. I suspect this book will have wide appeal, and not only to those familiar with the research world who might be interested in the details of this scandal. Those who think that research is still confined to the ivory tower will have their view broadened, and those concerned with today's changing moral standards will get an insight into forces that may influence behavior.

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Radiology of the Orbit, by Glyn A. S. Lloyd, 228 pp, with illus, $22.50, Philadelphia: W. B. Saunders Company, 1975

This monograph blankets nine modalities that are currently available for imaging the orbit. These modalities include noninvasive studies such as plain film examination, multidirectional tomography, A and B mode ultrasonography, and computerized tomography (CT), as well as invasive techniques such as venography, carotid angiography, pneumography, positive-contrast orbitography, and dacryocystography. Considering the choices available, it seems reasonable that certain anatomic structures would be best depicted by a specific examination. Lesions involving or arising within the optic globe are often best demonstrated by B mode ultrasonography, whereas those involving the remainder of the orbit are best visualized by accepted and recently innovated radiologic modalities. Most techniques reveal either the bony orbital confines or the orbital structures that can be opacified by contrast medium. Orbital soft tissues, optic nerve and globe, extraocular muscles, and orbital fat were not well displayed until CT was introduced.

Emphasizing unilateral ocular proptosis, the text is concise, well organized, and well illustrated. Chapters concerning vascular malformation and tumors deserve special attention because each lesion, including its basic pathologic process and usual site or sites of origin in the various surgical compartments, is discussed in depth. In addition, the author describes those radiographic, venographic, and angiographic features that allow a lesion to be localized to a compartment. The acme of this presentation is the author's illuminating approach to the analysis of orbital venography. The remainder of the treatise is devoted to orbital trauma, dacryocystography, and disease of the paranasal sinuses and nasopharynx.

Paradoxically, CT, which occupies only 8 pages of text, casts an ominous shadow over invasive radiologic techniques. One must heed the author's conclusion that CT will affect, if not replace, several currently accepted imaging modalities. The probability of replacement depends on the relative merit and inherent risk of each examination. Specifically, orbital pneumography and positive contrast orbitography seem vulnerable, while carotid angiography and orbital venography may remain viable since the intraorbital vascular anatomy is not well depicted by CT.

Conceptual changes in radiology are unusual, yet CT is currently shaking the pillars of medical imaging. During such a transitional period, major texts, that would have been widely circulated, may virtually be ignored. The precise role of CT is yet to be determined; however, a fundamental understanding of orbital lesions—the foundation of this monograph—is pertinent to any imaging method. Therefore, radiologists and other physicians interested in orbital diagnostics will benefit from reading this book.

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Handbook of Physiology, Section 7: Endocrinology, Volume VII—Parathyroid Gland, edited by Roy O. Greep, Edwin B. Astwood, and Gerald D. Aurbach, 488 pp, with illus, $55, Washington, DC: American Physiological Society, 1976

Seventh and last of the volumes on endocrine topics from the ongoing series of *Handbooks of Physiology*, its scope goes far beyond what its title implies. Only 5 of its 19 text chapters are directly concerned with the parathyroid glands and their secretory product(s). Actually, the contents span the entire field of mineral homeostasis in man and animals.

The editors have divided the topic into three broad areas: skeletal tissue and mineral homeostasis (10 chapters), parathyroid glands (5 chapters), and ultimobranchial glands and calcitonin (4 chapters). The 34 authors, almost all established investigators in their respective fields, provide individual chapters as diverse as "calcium homeostasis in cattle," "ionic control of metabolism," and "secondary hyperparathyroidism." The quality and readability of chapters vary enormously. I cannot agree with the advertising flyer accompanying the book, which claims that "this latest edition goes far beyond more conventional presentations in confronting the singular problems and controversial issues related to an understanding of . . . calcium metabolism." Bosh. These are mostly pedestrian but competent and thorough reviews of the usual sort. The authors, albeit experts, have biases that are plainly evident.

Some efforts must be praised: Raisz has produced a gem—brief, clear, and logical—on "mechanisms of bone resorption." This model should have been emulated by the other authors. Livingston and Wacker offer a provocative, concise, and useful review of that often-forgotten ion, magnesium ("Magnesium is *the* metal of *most general importance* in biochemical reactions. . . ." [emphasis added]). DeLuca once again skillfully and authoritatively reviews the recently revitalized vitamin D. In contrast, a few chapters deserve no praise: Glimcher's "composition, structure and organization of bone and other mineralized tissues and the mechanism of calcification" (longest title and longest chapter!) is prolix, dense, and underillustrated. Important issues are lost in the thick underbrush of verbiage. This chapter will be of great interest only to those intimately involved in related research.

A most egregious flaw is the failure of either chapter on bone to define or depict the osteon (or haversian system), the basic structural unit of compact bone. The naive reader could get a mistaken idea of the microstructure of bone from these presentations.

A few minor cavils. the lastest references are dated 1974. Therefore, many statements are out-of-date, and important advances in knowledge are omitted (such as Brewer's revised bovine PTH sequence, pre-pro-PTH, radioimmunoassay of calcitonin in normal human plasma, and immunoheterogeneity of circulating calcitonin). Of the 22 geographic areas represented by the authors, 16 are in the Eastern United States (most from Boston or Bethesda); this is reflected at points in some unevenness in choice of references. Secondary hyperparathyroidism rates a whole chapter whereas primary hyperparathyroidism is scarely mentioned. Munson is permitted to use the term "thyrocalcitonin" (current only in North Carolina) whereas all other authors in the book use the more usual "calcitonin." This variant usage goes unexplained in his otherwise admirable chapter.

This book will not be of value to clinicians. Its slightly "dated" character and high price will keep it from the shelves of many of the specialists who could use it; academic medical libraries must have it, of course. It will stand as an imposing monument to the "state of the art" of mineral metabolism in 1974.

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Explorations in Child Psychiatry, edited by E. James Anthony, 519 pp, $25, New York: Plenum Press, 1975

Anthony, a scholarly and prolific writer, has edited an important book for child psychiatrists. Child psychiatry is young enough as a medical specialty not to have accumulated a research tradition. Summing up the state of the art and science, Anthony deplores the lack of research in this specialty, which has remained chiefly service oriented. He states, "The art has flourished, but the science has stood still. . . .We must have more solid and verifiable underpinnings of knowledge than we currently possess." Despite this pessimistic assessment of the

present, he states that the purpose of the book is to be encouraging. It can be a stimulus for ideas and programs that will lead to the establishment of a firmer research foundation in the field.

To this end, he invited the contributions of 16 child psychiatrists active in a broad spectrum of research endeavors. Their essays are highly personal enunciations of their investigations, influences on their careers, and their philosophies. The contributors include both seasoned workers, whose wisdom is derived from experience, and younger investigators, whose fresh ideas hold much promise for the future. It is noteworthy that each of the contributors is first a clinician. Many had "backed into research" because of the unanswered questions that confronted them in their clinical work.

Chess, in describing the beginning of her career, writes that she assumed that all researchers started their investigations with elegant, unbiased designs, carried them through with single-mindedness of purpose, and finished with clear, unequivocal, meaningful results. She learned that the clinician is a practical person. To him falls the task of matching theory to actuality. Bruch's essay on the constructive use of ignorance epitomizes the necessity for open-mindedness, intellectual honesty, and clear thinking in an investigator.

The nature of the individual researchers emerges as the overriding influence that nurtured the spark of their investigational interest. This was further fostered by the models they saw in their teachers. Reading the book and thereby glimpsing the workings of some of the most active minds in the field gives one reason for optimism, for among the writers are the teachers of the present and next generation of child psychiatrists. The book is highly recommended.

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Pernicious Anemia (Major Problems in Internal Medicine, Volume VII), by Lawrence Kass, 261 pp, with illus, $17, Philadelphia: W. B. Saunders Company, 1976

In his monograph on pernicious anemia, Kass has brought together in a clear and interesting manner an account of the history and development of this fascinating syndrome. In dissertations of this type, the account of the historical aspects often consists of a few paragraphs to project the reader into the more meaningful text. In contrast to this technique, Kass has developed one of the most interesting historical accounts with which I am familiar. Not only is the work of the six Nobel laureates presented but also the contributions of many other individuals are well documented.

The author does a real service in bringing together and correlating an extensive literature on the chemistry, physiology, pathology, and pathogenesis of this megaloblastic anemia. The book is well illustrated, although the reader would appreciate having a few more of the prints published in color. Kass concludes with an epilogue presenting a number of problems that have concerned hematologists for years and are yet unsolved.

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FASEB Monographs, Volume 1, The Science of Life: Contributions of Biology to Human Welfare, edited by K. D. Fisher and A. U. Nixon, 384 pp, with illus, $27.50, New York: Plenum Press, 1975

In spite of an acknowledged political purpose, this volume succeeds in conveying an accurate account of many advances in medicine, dentistry, food science, population biology, marine science, and environmental sciences. For the most part, the chapters read smoothly and the examples are well chosen. Some inevitable redundancies do occur and, occasionally, the writing becomes too technical for the intended reader. But these are minor criticisms.

The major question that the work raises in my mind is whether it might have been more effective to emphasize the interrelatedness of "basic" and "applied" research and the contributions of research in one field to applications in another. The present volume touches upon these relationships but its primary focus remains on applications "to human welfare." Perhaps the task force responsible for the present compilation will consider emphasizing more fundamental concepts in a future volume.

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Fundamentals of Vascular Radiology, by Robert I. White, Jr., 163 pp, with illus, $13, Philadelphia: Lea & Febiger, 1976

Students, residents, and indeed any physicians involved or interested in angiographic procedures will greatly appreciate this text. It is complete enough to provide the necessary preangiographic evaluation, fundamental enough to help students get started, and thorough enough to alert the reader to areas of caution and potential hazards.

The principles of percutaneous catheterization and the equipment for special-procedure roentgenography are adequately done. These sections appropriately do not include any reference to coronary arteriography. The chapter on contrast material is well done and this, as well as all other chapters, is followed by an excellent bibliography. And the section on hemodynamic measurement is especially appropriate because this is an area often treated superficially during the usual rotation of a resident through vascular radiology. Each of the major areas of vascular anatomy, excluding coronary, is then reviewed quite adequately.

The chapter on pulmonary angiography is excellent, reflecting the author's experience and interest. And the final chapter is on pediatric angiography, an area many of us do not have extensive experience with; it seems to consolidate technical thinking in this area.

I would offer only a few minor criticisms of the text and will not comment on the techniques of the author, because technique is often merely a matter of individual preference. Perhaps a section could have been included about technical film quality because excellent anatomic display is needed for accurate diagnosis. The section on renal angiography perhaps should emphasize more the need for selective studies. There should always be as much anatomy as possible displayed when doing a flush or midstream aortogram, and I would not recommend end-occluded catheters.

This book meets the author's intent of providing the fundamental technical information needed to perform good angiography. It demonstrates his knowledgeable background in cardiology and radiology and underscores the necessity for any radiologist performing angiography to be fully conversant with the patient's problem and to be truly the consultant in charge of the examination and responsible for the patient's welfare.

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Venous Surgery in the Lower Extremities, edited by Lt. Col. Kenneth G. Swan, 464 pp, with illus, $32.50, St. Louis: Warren H. Green, 1976

Venous Surgery in the Lower Extremities is an assemblage of 34 papers interspersed with seven discussion sessions. Of the 19 participants in the symposium, 9 were from the military and 10 were civilians. The stated reason for holding the symposium was because this aspect of peripheral vascular surgery, especially due

to trauma, is not receiving the emphasis that it warrants today. The invited participants, most of whom are well-known in the field of vascular surgery, were chosen because of their ongoing interest in research; thus the inclusion of considerable laboratory data in the program was inevitable.

The clinical data on vascular and venous injuries, as presented in the first group of papers, were drawn mostly from the experiences of World War II, the Korean conflict, and the Vietnam conflict. The Vietnam experience indicated that the most common venous injuries involved the superficial femoral veins, the popliteal veins, and the brachial veins, in that order of frequency. Except for iatrogenic venous trauma, most venous injuries have concomitant arterial injuries and most of these injuries occur in the extremities. Of femoral artery injuries, at least 30 to 40% will have concomitant venous damage.

Various methods of measuring blood flow and the limitations of these methods are presented. The neurohumoral and exercise influences on blood flow in the extremity are discussed, and measures to prevent thrombosis in the injured veins by the meticulous attention to technical detail in the repair, by altering the blood velocity, by creating arteriovenous fistulas, and by using anticoagulants are included in other presentations.

A more apt title to this volume might be "A Symposium on the Physiologic and Surgical Aspect of Veins in the Lower Extremity Injured by Trauma." According to the title as stated, the book should otherwise have included the more commonly performed surgery for the veins of the lower extremity, such as the surgical treatment of varicose veins and the surgical treatment of acute superficial thrombophlebitis. Because the volume is a collection of papers presented at a symposium and not a textbook, there is an overlap of material presented and some recurring sameness in the bibliographies.

The gist of the symposium is a plea that the veins of the lower extremity, if injured, lacerated, divided, or torn, should (if at all possible) be repaired by direct suture or by interposing an allograft in hopes of restoring venous return. Data from the armed forces document this concept, which was supported by the experimental laboratory data. The importance of meticulous attention to technical detail in the repair of the vein, the use of anticoagulants, and the possible use of an arteriovenous fistula to accelerate blood flow across the site of repair are important factors in maintaining the patency of the vein following repair. This volume gathers together the current efforts in the clinical management of venous trauma and provides an overview of recent investigations of maintaining venous patency following repair. The symposium is of interest to the vascular research laboratory scientist more than to the practicing clinician, who is more often faced with the mundane challenges of the postphlebitic leg syndrome (which is only briefly mentioned) and the problem of varicose veins and varicose ulcers (which were not considered).

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Atlas of Cerebrovascular Disease, by William F. McCormick and Sydney S. Schochet, Jr., 428 pp, with illus, $28, Philadelphia: W. B. Saunders Company, 1976

Anatomic pathologic studies represent the basis for ultimate diagnosis in most disorders, but particularly in vascular diseases of the nervous system. This volume is largely a photographic atlas of the normal and abnormal anatomy of the blood vessels as well as of the resulting nervous system parenchymal lesion related to blood vessel disorders.

The sections on arterial and venous anatomy are conventional descriptions but include excellent black and white photographs emphasizing the arteries at the base of the brain. In addition, there are photographs with line drawings indicating the regions perfused by the various blood vessels that are described, and this format is helpful in developing visual images of the relationships of artery and appropriate brain parenchyma.

It is true for the section on ruptured aneurysms, as it is for other sections, that the photographic material presented represents a distortion of the disease process, simply because the studies were taken from autopsy collections. The book, therefore, presents a disproportionate representation of severe problems that more commonly lead to death. This may be particularly notable in respect to the section on subarachnoid hemorrhage from aneurysms and angiomas or arteriovenous malformations.

The authors cover the clinical effects of systemic hypertension somewhat sketchily but raise appropriate questions about the precise relationship between systemic hypertension and intracerebral hemorrhage. There is also, in the section on occlusive disorders, an excellent description of the technique for removing extracranial arteries at autopsy. There are also a valuable description and excellent photographs demonstrating the consequences and location of lesions in the carotid artery that occur associated with intramural injections at the time of arteriography.

The discussion on arterial and venous thrombosis in patients who are on oral contraceptives is not a very critical one, and no evidence is presented for a causal relationship between the lesions noted and the administration of oral contraceptives. Furthermore, there is no discussion of thrombosis and infarction in young persons in general in circumstances in which there is no known etiologic precipitating mechanism.

As in the description of cerebral infarctions, the authors also use brief case histories as the introduction to presenting photographs of pathologic material in various types of brain hemorrhage. In raising appropriate questions about the relationships between hypertension and brain hemorrhage, the authors on several occasions erroneously use the word "prevalence" when they actually mean frequency of finding a lesion or a condition at autopsy examination. They emphasize that the causative factors in intracerebral hemorrhage are unknown and they appear to favor the old idea, not yet disproven, that hemorrhage occurs primarily in areas of previous infarction. In addition to a brief section on intracranial hemorrhage related to ruptured aneurysms, there is a short description of numerous other conditions that might be associated with intracranial hemorrhage.

The section on mass lesions adds little and simply points out that when aneurysms, arteriovenous malformations, or ectatic arteries are adjacent to cranial nerves or other parts of the central nervous system, they may cause dysfunction by pressure on those portions of the nervous system.

The chapter on spinal cord infarction is superfluous because it is not handled very completely and only covers spinal cord infarction related to aortic disease. There is no comprehensive discussion of how various distributions of spinal cord infarction occur. Because the title of the book relates to cerebral vascular disease, it would not have been a loss to have omitted this chapter.

The excellent photographs in this atlas are the primary value of the work. They are organized along the lines of clinical description to provide an easy reference to clinicopathologic correlations of cerebral vascular disorders.

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Cooking for Diabetics at Home and Away, by Winnie Balfour Rhodes, 261 pp, $12.50, Springfield, Illinois: Charles C Thomas, Publisher, 1976

Much more than a cookbook, this book contains many hints that will ease the daily routine of the diabetic person and his family. Almost all dietary circumstances are anticipated. For instance,

there is even a chapter on being a house guest. The difficulties of adhering strictly to a prescribed diet are recognized, and many options are given for lessening any feeling of monotony while not straying too far from the dietary regimen. The use of artificial sweeteners is considered in a rational way with specific instructions and examples.

The author states that the recipes have been tested repeatedly, and their formulations give the appearance that this is so. A strong plea is made for baking one's own bread for the greater enjoyment of our staff of life. Certainly, among the taste sensations available to us, freshly baked bread rates among the most delectable. One might question the use of artificial sweeteners in some of the bread recipes, since the sugar added in most bread recipes is fermented by the yeast and thus is not to be considered as a form of sugar in the diet. The authors use potato as a substrate for the yeast and the artificial sweeteners are thus intended for flavor.

Most of the recipes are for family-sized servings and hence will find ready use. Conventional household measures are used throughout, and some arithmetic would be needed for the cook who might "go metric" in the next several years.

It is unfortunate that this excellent cookbook has been published at the time the new exchange lists have been published by the American Diabetes Association and the American Dietetic Association. These new exchange lists are sufficiently different from those used in this cookbook so that a given recipe may yield appreciably different numbers and sorts of exchanges by the new scheme. The qualified dietitian and the diabetic person who knows his dietary principles thoroughly can make the necessary translation from the new to the old exchanges, however.

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Self Assessment in Clinical Cardiology, II, edited by Michael S. Gordon, 363 pp, with illus, $11.95, Chicago: Year Book Medical Publishers, 1976

This second volume was patterned after the first and, like it, was derived from a teaching conference in clinical cardiology held at the University of Miami. This origin lends strengths but also some weaknesses. There is a kind of spontaneity and variety to the subjects that are derived from the different contributors, but the strength of their written contributions varies. This variation might not be an accurate measure of the presentations at the teaching conference. The editor attests that he has expended considerable effort in editing the material. In the list of subjects derived from a teaching conference the horizon is necessarily somewhat limited; the list of topics in this book seems to reflect this bias rather than a situation in which the editor included everything he considered important.

Cardiology requires basic information as well as the latest clinical advances and items of classical bedside teaching. All these Gordon has included. Among the most difficult subjects to write about and to test are those that usually are imparted verbally and that usually are included as classical bedside teaching. In this book the effort to teach and to test are both made and the result is quite acceptable. There is a subtle inducement to learn created by the title and by the two main parts. The title suggests that each reader is being asked to compete with himself and thereby to enrich his knowledge. The instructional portion would be expected to impart the knowledge that the second part tests. In fact, both parts teach. The testing section has answers that can be seen, disputed, and evaluated, and the test becomes a kind of cap on the learning experience.

How about the future? I hope that Gordon will continue—ideally, using the same kind of outline, possibly expanded. The subject list might focus on a broader spectrum of what should be understood in cardiology, either in terms of a core of knowledge or in terms of "recent developments." There is a fine conceptual line between a thorough review book and a definitive textbook. This book is not yet a really thorough review but it has promise and form. It is well worth the time of the interested reader to obtain the book and enlarge with the editions.

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Scientific Foundations of Oncology, edited by T. Symington and R. L. Carter, 710 pp, with illus, $65, London: William Heinemann Medical Books Publication, 1976

The editors emphasize the importance of communication between clinical and nonclinical oncologists and propose to facilitate this communication by presenting "authoritative summaries of modern trends" in oncology. The book contains 73 such summaries, or chapters, by 101 authors. The main sections consist of pathology, biochemistry, cell kinetics, chromosomes, invasion and metastases, genetics, epidemiology, carcinogenesis, immunology, systemic effects of neoplasia, recent developments in diagnosis, and current problems in treatment.

The section on pathology includes concise, provocative examinations of topics ranging from ultrastructure of tumor cells to tissue culture methods and cell fusion studies. The general format for the sections on biochemistry, epidemiology, and immunology is that of an initial general discussion followed by a detailed examination of the appropriate discipline in several specific neoplastic diseases. The consideration of carcinogenesis covers a large portion of the book, about one-third, and is comprehensive and particularly well done.

The coverage of treatment aspects is relatively superficial, not receiving the same degree of attention as do many of the other subjects in the book. The chemotherapy of breast cancer, lung cancer, and the lymphomas are each covered in one-half page or less, that of colorectal cancer in about one-quarter page, and melanoma, one sentence. The discussion of surgical treatment is concerned with generalities and philosophy of approach rather than with a thorough analysis of treatment of specific disease entities. The authors' intent appears to be the presentation of an overview of different modalities of treatment, and this is not a book that the practicing doctor will find particularly helpful for determining therapy for the individual patient.

As with any multiauthored book, the chapters vary in quality, but overall the quality is high. Most of the authors are European and, as would be expected, there is an emphasis on the citation of European literature. The book is, in general, quite good and will be found to be useful to those individuals with a special interest in neoplastic diseases, from the medical and graduate student to the oncologist, whether clinical or nonclinical.

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BOOK NOTICES

Adventures in Medical Research: A Century of Discovery at Johns Hopkins, by A. McGebee Harvey, 480 pp, with illus, $16.95, Baltimore: Johns Hopkins University Press, 1976

A certain danger invests any attempt to describe the outstanding accomplishments of an institution. The danger, of course, is chauvinism. Harvey escapes the trap by letting the facts tell the tale. And a remarkable tale it is: of investigators such as Opie, Cullen, Mall, Florence Sabin, Hammon, Rich, Longcope, Blalock, Perrin Long, and dozens of others. The book is readable, well documented, and fascinating.

Epidemic and Peace, 1918, by Alfred W. Crosby, Jr., 343 pp, with illus, $17.50, Westport, CT: Greenwood Press, 1976

As Crosby points out, the catastrophic influenza epidemic of 1918 has received remarkably little attention by historians. He has attempted to remedy this deficiency. The book is well written and thoroughly documented; its coverage is not only medical but also political, social, and military. The timing of publication seems especially apt because of the current predictions of an upcoming epidemic of swine influenza.

Eugenics: Then and Now (Benchmark Papers in Genetics, Vol. 5), edited by Carl Jay Bajema, 400 pp, with illus, $25, Stroudsburg, PA: Dowden, Hutchinson & Ross, 1976

For the 110 years since Galton invented the word, eugenics has been the arena for attacks and counterattacks of the most vigorous—sometimes vicious—variety. Within this volume, Bajema has collected much of the most pertinent literature of that lengthy (and certainly unended) controversy. Of all the similar volumes produced by this publisher, *Eugenics* seems to have the most direct and continuing relevance, for the emotional issues are unchanged and science has done little to alter these issues.

American Medical Education (The Formative Years, 1765-1910), by Martin Kaufman, 219 pp, $12.95, Westport, CT: Greenwood Press, 1976

Some coherent picture of the growth of medical education in this country has long been needed. The process did *not* begin with Flexner; indeed, that is where this book ends. Kaufman traces the growth through the days of the empirics, the problems of grave-robbing, the rise of medical sects, and the shattering political struggles that went on seemingly endlessly. Certainly the good old days were not so splendid.

BOOKS RECEIVED

Fundamentals of Clinical Hematology, 4th ed, by Byrd Stuart Leavell and Oscar Andreas Thorup, Jr., 769 pp, with illus, $25, Philadelphia: W. B. Saunders Company, 1976

The Healing Hand: Man and Wound in the Ancient World, by Guido Majno, 597 pp, with illus, $25, Cambridge: Harvard University Press, 1976

The Cerebral Vessel Wall, edited by J. Cervós-Navarro, E. Betz, F. Matakas, and R. Wüllenweber, 287 pp, with illus, $22, New York: Raven Press, 1976

Color Atlas & Textbook of Tissue and Cellular Pathology, 5th ed, by Walter Sandritter, 329 pp, with illus, $29.95, Chicago: Year Book Medical Publishers, 1976

Advances in Pediatrics, Volume 2, edited by Irving Schulman, 421 pp, with illus, $27.50, Chicago: Year Book Medical Publishers, 1976

A Laboratory Guide to Clinical Diagnosis, 4th ed, by R. D. Eastham, 311 pp, $8.95, Bristol: John Wright & Sons, 1976

Color Atlas of General Surgical Diagnosis, by William F. Walker, 448 pp, with illus, $37.95, Chicago: Year Book Medical Publishers, 1976

A Short Practice of Clinical Psychiatry, by Russell Barton, 421 pp, with illus, $29.95, Bristol: John Wright & Sons, 1975

Blood-Brain Barrier in Physiology and Medicine, by Stanley I. Rapoport, 328 pp, with illus, $25, New York: Raven Press, 1976

The Language of Medicine: A Worktext Explaining Medical Terms, by Davi-Ellen Chabner, 653 pp, with illus, $11.50, Philadelphia: W. B. Saunders Company, 1976

Hypothalamic-Pituitary-Adrenocortical Regulation: A Contribution to Its Assessment, Development and Disorders in Infancy and Childhood with Special Reference to Plasma Cortisol Circadian Rhythm (Monographs in Paediatrics, Vol. 7), by Rolf P. Zurbrügg, 91 pp, with illus, $20, Basel: S. Karger AG, 1976

The Neuropsychology of Memory, by Alexander R. Luria, 388 pp, with illus, $22.95, Washington, D.C.: V. H. Winston & Sons, 1976

The Heart and Circulation (Benchmark Papers in Human Physiology, Vol. 8), edited by Peter A. Chevalier, 407 pp, with illus, $26, Stroudsburg, PA: Dowden, Hutchinson & Ross, 1976

The Manpower Problem in Mental Hospitals: A Consultant Team Approach, by Philip F. D. Seitz, Elizabeth Jacob, Harold Koenig, Ruth Koenig, Warren G. McPherson, et al, 266 pp, $12.50, New York: International Universities Press, 1976

Dental Biochemistry, 2nd ed, edited by Eugene P. Lazzari, 400 pp, with illus, $16.50, Philadelphia: Lea & Febiger, 1976

Introduction to Pharmaceutical Dosage Forms, 2nd ed, by Howard C. Ansel, 423 pp, with illus, $22.50, Philadelphia: Lea & Febiger, 1976

Pharmacognosy, 7th ed, edited by Varro E. Tyler, Lynn R. Brady, and James E. Robbers, 547 pp, with illus, $21.50, Philadelphia: Lea & Febiger, 1976

Spinal Cord Injuries: Comprehensive Management and Research, 2nd ed, by Professor Sir Ludwig Guttmann, 747 pp, with illus, $65, Oxford: Blackwell Scientific Publications, 1976

Acute Diarrhoea in Childhood, Ciba Foundation Symposium 42, 385 pp, with illus, $26.95, Amsterdam: Elsevier/Excerpta Medica/North-Holland, 1976

Manual on Control of Infection in Surgical Patients, by American College of Surgeons Committee on Control of Surgical Infections of the Committee on Pre- and Postoperative Care (William A. Altemeier, Chairman), 313 pp, with illus, $16, Philadelphia: J. B. Lippincott Company, 1976

The Diagnosis of Bleeding Disorders, 2nd ed, by Charles A. Owen, E. J. Walter Bowie, and John H. Thompson, 414 pp, with illus, $21.50, Boston: Little, Brown & Company, 1975

Ophthalmic Electrodiagnosis (Major Problems in Ophthalmology, Vol. 1), by N. R. Galloway, 179 pp, with illus, $21.50, London: W. B. Saunders Company, 1975

Health: A Victim or Cause of Inflation?, edited by Michael Zubkoff, 416 pp, with illus, $4 (paperback), New York: Prodist, 1976

Dynamic Radiology of the Abdomen: Normal and Pathologic Anatomy, by Morton A. Meyers, 392 pp, with illus, $29.80, New York: Springer-Verlag, 1976

Fundamentals of Urology, by Jack Lapides, 609 pp, with illus, $17.50, Philadelphia: W. B. Saunders Company, 1976

Present Diagnosis and Treatment of Septicemia (Antibiotics and Chemotherapy, Vol. 21), edited by L. P. Garrod, H. Seneca, E. Jawetz, and J. Fereres, 224 pp, with illus, $50.50, New York: S. Karger, 1976

Practical Ophthalmic Plastic and Reconstructive Surgery, by Merrill J. Reeh, Charles K. Beyer, and Gerard M. Shannon, 215 pp, with illus, $24.50, Philadelphia: Lea & Febiger, 1976

Esophageal Hiatus Hernia: Rationale and Results of Anatomic Repair, by Thomas Gahagan and Conrad R. Lam, 205 pp, with illus, $19.50, Springfield, IL: Charles C Thomas, Publisher, 1976

Synthesis of Life (Benchmark Papers in Organic Chemistry), edited by Charles C. Price, 405 pp, with illus, $22, Stroudsburg, PA: Dowden, Hutchinson & Ross, 1974

Glomerulonephritis (Vol 2, Contributions to Nephrology), edited by R. B. Sterzel, D. Thomson, and J. Brod, 200 pp, with illus, $20.75, New York: S. Karger, 1976

Total Parenteral Alimentation: Proceedings of the International Symposium on Intensive Therapy, Rome, May 30-June 2, 1975, edited by C. Manni, S. I. Magalini, and E. Scrascia, 344 pp, with illus, $36.50, New York: Excerpta Medica, 1976

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Management and Prognosis of Multiple Myeloma

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Patients with asymptomatic or smoldering multiple myeloma should not be treated but should be observed closely for progression. For symptomatic myeloma, chemotherapy is indicated. Melphalan, the agent of choice, should be given with prednisone for 1 week of every 6 weeks. If melphalan brings no response, or response and then relapse, cyclophosphamide (Cytoxan) should be given intravenously every 4 weeks or orally every day. BCNU, CCNU, and doxorubicin (Adriamycin) have also shown activity in myeloma. Hypercalcemia occurs in one-third of patients and should be countered with hydration, corticosteroids, Neutra-Phos, or mithramycin. Long-term hemodialysis has achieved some success. The combination of sodium fluoride and calcium carbonate produces new bone formation; it seems a useful adjunct in treatment for myelomatous bone disease. Radiation should be utilized only for severe, localized pain or for solitary lesions. Survival with multiple myeloma varies, mean durations being 2 to 3 years. Multivariate analysis indicates that serum creatinine and calcium levels are the most significant indicators regarding 2-year survival. We have found monoclonal proteinuria not significantly more frequent with renal insufficiency than with normal renal function, renal insufficiency not significantly more frequent with lambda than with kappa chains, and survival not significantly greater with IgG myeloma than with IgA.

Most patients with multiple myeloma have symptoms or laboratory evidence of significant disease at the time of diagnosis and clearly need to be treated. However, some patients have no symptoms and only moderate laboratory abnormalities, and in these cases it may be difficult to distinguish multiple myeloma from benign monoclonal gammopathy. This presents a dilemma to the clinician, because the greater the myeloma cell mass in a patient, the less likely a satisfactory response to chemotherapy. Conversely, if the patient is tolerating his disease well, one does not wish to burden him with the inconvenience, expense, and possible side effects of chemotherapy. Furthermore, it conceivably could increase the activity of the myeloma. In one instance a monoclonal gammopathy remained static for 5 years while it was untreated but then, when cytotoxic drugs were given, the protein abnormality increased and the patient showed clear evidence of rapidly advancing malignancy.[1]

Patients in whom it is difficult to distinguish between multiple myeloma and benign monoclonal gammopathy and those who have a low-grade, smoldering myeloma should *not* be treated but should be reevaluated at

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intervals of 3 to 6 months. If clinical and laboratory examinations show no progression of the disease, the same program of observation should be continued without treatment. My colleagues and I have followed up one such patient without therapy for more than 10 years. In this case, an unequivocal diagnosis of myeloma had been made on the basis of bone-marrow morphology and a monoclonal serum protein concentration of 3 g/dl; but these findings have not changed and other features of myeloma have not developed during the 12-year observation period.

CHEMOTHERAPY

Chemotherapy is the best initial treatment for multiple myeloma, unless there is disabling pain that is clearly the result of a well-defined focal process. If analgesics together with chemotherapy can control the pain, chemotherapy is much preferred to repeated local irradiation, because the bone-marrow reserve of many myeloma patients is limited and irradiation does not benefit systemic disease.

Alkylating Agents. Melphalan.—Melphalan (L-phenylalanine mustard, L-sarcolysin, Alkeran) is an alkylating agent with an active nitrogen mustard radical attached to the amino acid, L-phenylalanine. Synthesized in 1953 and introduced 5 years later,[2] melphalan has become well established in chemotherapy for multiple myeloma.[3-5]

Melphalan has been used successfully in a low-dose continuous regimen producing objective benefit.[6,7] An intermittent schedule was reported superior to daily therapy;[8] but in another study pain recurred before the next cycle of therapy, and consequently the authors felt that continuous maintenance was superior to intermittent therapy.[9] In a prospective randomized study, comparisons were made between effects of (1) a loading dose of melphalan followed by daily oral therapy, (2) the same melphalan schedule plus prednisone in a decreasing dose for 10 weeks, and (3) then the same schedule of melphalan and prednisone plus testosterone enanthate. Melphalan plus prednisone produced a good response in 55%, versus 23% for those receiving melphalan only. Median survival, for good-risk patients who responded, for the prednisone-melphalan group was 53 months, versus 30 months for melphalan alone.[10]

Melphalan is the drug of choice in treatment for myeloma. We prefer to give it intermittently with prednisone, because the benefits appear to be better than with melphalan alone in a daily oral schedule. Fewer blood counts and patient visits are necessary to maintain it. We give 0.15 mg/kg orally each day for 7 days. For the average-sized person, this is equivalent to 8 to 10 mg daily, or a total dose of 56 to 70 mg for 1 week. Prednisone in a dosage of 15 mg four times daily is given for the same 7-day period. While prednisone is being given, the patient should have a bland diet and take antacids. The patient is reevaluated at 6-week intervals for consideration of further courses of melphalan and prednisone. Leukocyte and platelet counts must be obtained prior to therapy, as leukopenia and thrombocytopenia often occur.

The subsequent courses of melphalan may need to be altered: if the leukocyte and platelet counts have not decreased, the daily dose of melphalan may be increased by 2 mg daily for the next 7-day course; whereas if the counts have decreased, the dose of melphalan should be lessened accordingly. Renal insufficiency may be an indication for using a smaller dose and being cautious, for melphalan is excreted by the kidneys. However, we have not been impressed with excessive hematologic toxicity of melphalan in the presence of significant degrees of renal insufficiency. The dose of prednisone ordinarily would not be altered unless the patient has an active peptic ulcer, severe hypertension, or uncontrolled diabetes. Occasionally a patient has withdrawal symptoms; if so, we reduce the prednisone to 0 over a period of 4 to 5 days rather than stop it abruptly at the end of the 7-day course.

The patient should be given at least three 7-day courses before this schedule is abandoned, unless it causes significant toxic reactions or the disease progresses rapidly in spite of clearly adequate therapy. Maximal improvement may not be achieved for many months. The appropriate duration of chemotherapy is unknown. In a recently reported study, 96 patients responding to chemotherapy at 1 year were randomized to three groups: (1) melphalan plus prednisone every 6 weeks, (2) carmustine (BCNU) plus prednisone, and (3) no treatment. There was no difference among these groups in the frequency of relapse, the length of remission, or the survival time—suggesting that chemotherapy need not be continued beyond the first year if the patient responds to treatment.[11] However, we prefer to continue chemotherapy as long as any evidence of myeloma exists or unless other investigators confirm the finding that maintenance therapy is not needed.

Melphalan may also be given in a daily oral dosage; the leukocyte and platelet counts are determined every 2 weeks, and the dosage of melphalan is adjusted to keep the leukocytes in the neighborhood of 3,000/mm^3 and the platelets between 100,000 and 150,000/mm^3.

The major side effect of melphalan therapy is hematologic—that is, bone-marrow suppression. Nausea, vomiting, pruritus, and skin rash develop occasionally but are seldom significant. Pulmonary fibrosis has been reported in a single instance.[12]

Cyclophosphamide.—If there is no response to melphalan, or if the patient responds to it and then becomes resistant, cyclophosphamide (Cytoxan) may be effective. Cyclophosphamide is an alkylating agent, a cyclic phosphoric acid ester derivative of nitrogen mustard. Oral administration of it in a dosage of 2 mg/kg body weight daily for long periods has brought improvement in at least 25% of patients with myeloma.[13] Because leukopenia and thrombocytopenia occur frequently, the dose is adjusted according to the leukocyte and platelet counts, which should be determined every 1 to 3 weeks.

Bergsagel and associates[14] reported good or partial responses to intermittent administration of cyclophosphamide in a single intravenous dose of 1.0 g/m² or oral doses of 0.25 g/m² daily for 4 days in 11 of 19 myeloma patients who had developed resistance to melphalan. Cyclophosphamide was administered every 3 weeks in both regimens. Our experience with intravenous administration of cyclophosphamide, 600 mg/m², plus prednisone every 6 weeks to patients resistant to melphalan has been less encouraging: good or limited responses were obtained in only 16 of 38 cases (42%).[15] A number of factors—including smaller doses of cyclophosphamide in our series and difficulties in defining resistance to melphalan and in evaluating response—hinder direct comparisons.

In addition to leukopenia and thrombocytopenia, side effects of cyclophosphamide include nausea, alopecia, hemorrhagic cystitis, fibrosis of the urinary bladder,[16] carcinoma of the urinary bladder,[17] and depression of ovarian[18] or testicular[19] function.

In two separate studies, the survival of patients treated with melphalan orally and of others treated with cyclophosphamide orally has not differed appreciably.[20,21]

BCNU.—BCNU (1,3-bis[2-chloroethyl]-1-nitrosourea) (carmustine) has proved to be beneficial and appears to be almost as effective as melphalan for previously untreated patients.[22] This drug is given intravenously every 6 weeks in a dose of 150 mg/m²; and the patient needs to be seen only at 6-week intervals. Its major side effects include nausea, vomiting, and bone-marrow depression.

Other Chemotherapeutic Agents.—First reported to be of benefit by Alwall[23] in 1947, urethan was the only chemotherapeutic agent for several years. Subsequent results have been less impressive than the initial results, and one controlled study showed that orally administered urethan was not superior to placebo therapy statistically and that in patients treated with urethan the survival was actually shorter.[24] However, there is no doubt that occasionally patients did respond to urethan. Urethan given intravenously may be of some benefit, but the results are not impressive and the toxic effects are significant.[25]

Other agents used for myeloma include tryptophan mustard,[26] 6-thioguanine,[27] a combination of 6-thioguanine and azaserine,[28] azathioprine (Imuran),[29] streptonigrin, procarbazine (Matulane),[30] mechlorethamine (nitrogen mustard), triethylenemelamine (TEM), 6-mercaptopurine, chlorambucil,[29] and aniline mustard.[31] Although possessing some activity, these agents are inferior to either melphalan or cyclophosphamide.

Doxorubicin (Adriamycin), used in a few cases of multiple myeloma, did not produce encouraging results as a single agent.[32] In another study, doses of 25 to 45 mg/m² of doxorubicin produced benefit in three of nine patients who had failed to respond to intermittent treatment with melphalan and prednisone. However, bone-marrow suppression was significant and the remissions were short.[33]

Another agent which has produced some objective benefit in myeloma is CCNU, which has the additional advantage of oral administration.[22]

Combinations of Chemotherapeutic Agents.—Combinations of melphalan, cyclophosphamide, BCNU, and prednisone, given intermittently, produced an impressive response in one group of 20 patients;[34] and melphalan, cyclophosphamide, prednisone, BCNU, and vincristine produced a response in 90% of another group of 36 patients.[35] Azam and Delamore[36] also reported encouraging preliminary results from giving BCNU, cyclophosphamide, melphalan, and prednisone to myeloma patients who had ceased to respond to single-agent therapy or who had not been treated previously. We have been unable to demonstrate, in a prospective, randomized study, that responses to melphalan, BCNU, cyclophosphamide, and prednisone together were better than responses to only melphalan and prednisone given orally. However, survival differences may become apparent.[22] Further evaluation of combinations of drugs in a prospective controlled fashion is necessary.

Addition of procarbazine to melphalan and prednisone increased the response to 59% from 48%. The survival time of all patients was similar in the two regimens, as was the length of remission of responding

patients. Moderate nausea and skin eruptions were more frequent in the group given procarbazine.[37] Thus this agent adds little to the management of multiple myeloma.

The combination of doxorubicin (Adriamycin) and BCNU, each in a dosage of 30 mg/m² given intravenously every 3 to 4 weeks, reduced tumor cells at least 50% in 7 of 13 patients who did not respond to or had relapsed after treatment with alkylating agents and prednisone. This combination appears promising and further studies of it are desirable. The total dosage of Adriamycin must be limited to 500 mg/m² because of possible cardiac toxicity.[38]

Side Effects.—Chemotherapy often produces cytopenia and usually causes leukocyte and platelet values to be low during its use. However, progressive invasion of the bone marrow by plasma cells may produce pancytopenia; in this situation bone-marrow aspiration and biopsy are necessary to determine whether the effect is due to such invasion or to chemotherapy.

Rapidly fatal acute myelomonocytic leukemia has developed in more than 40 patients with multiple myeloma who were treated with an alkylating agent.[39] Most of them had been given melphalan, usually on a daily schedule; but some received it intermittently, and a few had been given cyclophosphamide or radiation. We believe that the alkylating agents had a significant part in the development of the acute leukemia in these patients, but we cannot exclude the possibility that acute leukemia may occur during the natural course of myeloma. There have been several cases in which multiple myeloma and acute leukemia were found simultaneously. Although the development of acute leukemia and myeloma is probably related to alkylating agents, the incidence is extremely low and onset occurs only after a significant period of treatment, during which the patient is often in a remission he otherwise would not have had. There can be no question that the treatment of myeloma with alkylating agents is justified, despite the small risk of acute leukemia.

Criteria for Response.—The effects of therapy should be assessed at regular intervals by determining the concentrations of hemoglobin and of serum and urinary protein, examining the bone marrow, and evaluating skeletal roentgenograms. Favorable response to therapy can be judged by the following criteria: (1) increase of the hemoglobin concentration by 2.0 g/dl (without transfusion in a period of at least 4 weeks) from an initial value of less than 11.0 g/dl; (2) decrease of the monoclonal serum protein to 50% or less of the initial value; (3) decrease of the monoclonal urinary globulin to 50% or less—if the initial value was 1.0 g/24 h or more; (4) decrease of the number of plasma cells in bone marrow by 50% or more, as shown on repeated marrow aspirations; (5) recalcification of skeletal lesions and absence of new osseous lesions.

Subjective improvement, to be recognized as significant, should reflect an increase of performance by two grades or more on the following scale of activity: Patient is (1) normal; (2) ambulatory, has symptoms, but spends less than 50% of waking time in bed; (3) symptomatic and spends more than 50% of waking time in bed; (4) completely bedridden. The severity of pain may be graded from 0 to 4 (none to incapacitating; again, improvement by two grades to be significant). Increase in the concentration of the normal (uninvolved) immunoglobulin components has been reported as another useful indicator of response.[40] A proposed guideline for protocol studies for myeloma has been published by a Committee of the Chronic Leukemia-Myeloma Task Force, National Cancer Institute.[41]

OTHER APPROACHES TO THERAPY

The labeling index of myeloma cells indicates the fraction of cells in DNA synthesis and provides an estimate of the growth fraction of the cell population. Incubation of myeloma cells after chemotherapy reveals a high tritiated thymidine labeling index, indicating that an increased proportion of cells are proliferating during apparent remission. An S-phase specific agent, vincristine, has been reported to further decrease the number of myeloma cells.[42]

After initial reduction of the tumor-cell burden with chemotherapy, the patient might be given an immunotherapeutic agent such as BCG or MER (methanol-extractable residue of BCG).

Plasmacytomas of BALB/c mice may be inhibited by anti-idiotypic antibody. Both prevention of tumor growth and regression of plasmacytomas may be seen after injection of anti-idiotypic antibodies.[43] It is conceivable that injection of anti-idiotypic antisera might be beneficial in human myeloma.

In mice with MOPC 104E myeloma, prednisone and melphalan had a therapeutic effect; and BCNU, cyclophosphamide, and prednisone in combination had significant therapeutic effect but also significant toxicity.[44] Use of this tumor system in testing other drug combinations may be helpful.

Total-body irradiation has been suggested as treatment for myeloma. In a trial, 11 patients with myeloma were given 150 R while 10 others received a conventional course of melphalan orally. The mel-

phalan produced better therapeutic results with less toxic reaction than did the total-body irradiation.[45]

MANAGEMENT OF SPECIAL PROBLEMS

Hypercalcemia.—Hypercalcemia occurs in at least one-third of patients with multiple myeloma. To be recognized, it must first be considered; and it must be excluded in the presence of anorexia, nausea, vomiting, polyuria, increased constipation, weakness, confusion, stupor, or coma. When hypercalcemia is symptomatic, treatment is urgent. Hypercalcemia often leads to renal insufficiency, but prompt treatment often improves renal function.

Because dehydration is a common accompaniment, the patient with hypercalcemia should be hydrated. Saline is the agent of choice, since sodium promotes the renal excretion of calcium. The addition of furosemide (Lasix), 40 mg every 4 hours, may be beneficial. During diuretic therapy, the electrolytes must be monitored closely. In addition to hydration and diuresis, we give prednisone in an initial dosage of 100 mg daily. This must be reduced after a few days and discontinued as quickly as possible. Inorganic phosphate (Neutra-Phos) may be given with prednisone. We prescribe 150 ml orally four times daily (equivalent to 2.0 g of phosphorus daily). If the patient is comatose, the inorganic phosphate solution can be given via gastric tube or rectally. If he is critically ill, inorganic phosphate can be given slowly intravenously. If these measures fail, mithramycin should be given intravenously in a dose of 25 μg/kg body weight. It produces its effect within 24 to 48 hours, but hypercalcemia often recurs after 2 or 3 days. Thrombocytopenia may follow, so depression of bone-marrow function is a relative contraindication to the use of mithramycin.

Renal Insufficiency.—Uremia is one of the major causes of death in multiple myeloma. (Hypercalcemia is one of its most treatable causes and has been discussed above.) Hyperuricemia may contribute to renal insufficiency; but for hyperuricemia, allopurinol provides effective therapy. If the patient is allergic to this drug, alkalization of the urine with sodium bicarbonate, 0.6 to 0.9 g three or four times daily, and acetazolamide (Diamox), 250 mg at bedtime, is effective.

A trial of hemodialysis definitely is indicated in acute renal insufficiency and myeloma. We have had several patients in such condition who regained adequate renal function. We have treated eight patients with long-term dialysis, and the results have been encouraging; but its use is subject to availability and other local circumstances.

Infections.—Bacterial infections are frequent in myeloma and sometimes fatal. Significant fever is an indication for appropriate cultures, roentgenography, and consideration of antibiotic therapy. The choice of antibiotics can be more specific once the results of the cultures are known. There is considerable doubt as to the efficacy of gamma-globulin injections for preventing infection in myeloma. Occasionally we have used penicillin prophylactically with good results in cases of multiple, recurrent severe bacterial infections. Use of gentamicin for infections in patients with multiple myeloma must be carefully monitored; we occasionally have seen a marked decrease of renal function following small doses of the drug.

Neurologic Complications.—Extradural extension of myeloma may compress the spinal cord or cauda equina and thus produce progressive weakness and sensory loss leading to complete and permanent paraplegia. Although irradiation may be the best therapy,[46] the effect of ionizing radiation is rather slow; and if the neurologic deficit progresses during radiation therapy, surgical decompression is essential. One should not abandon consideration of therapy, even for the patient who is paraplegic from spinal-cord compression. We have seen a patient who had had symptoms of cord compression for more than 2 weeks prior to surgery—and had been unable to walk for 4 days—recover the function of his lower extremities after surgical decompression and radiation therapy. The ultimate prognosis should not unduly affect the decision to operate; patients prefer ambulatory survival for only a year rather than permanent paraplegia.

Skeletal Lesions.—Skeletal lesions with pain and fracture are a major problem in patients with myeloma. Although bone lesions are reported to heal with chemotherapy, we have been very disappointed in this regard. Frequently a brace or supporting garment is helpful; but avoidance of trauma is more important because even mild injury may result in multiple fractures. Nevertheless, the patient should be encouraged to be as active as possible, because confinement to bed increases demineralization of the skeleton. Analgesics should be given to control pain so that the patient can be ambulatory. Physical therapy also may be beneficial. Fixation of fractures of the long bones with an intramedullary rod and methyl methacrylate has given very satisfactory results in our experience.

We compared the effect of sodium fluoride, 50 mg twice daily with meals, and calcium carbonate, 1 g

Table 1.—**Bone Changes in Myeloma Patients Treated With Fluoride-Calcium or Placebo***

	No.	Before treatment, mean	No.	After treatment, mean
Fluoride-calcium				
Formation, %	10	3.6	12	9.0†
Resorption, %	5	8.1	4	2.4
Cortical thickness, mm	10	0.6	13	0.9‡
Trabecular thickness, μm	11	118.0	13	208.6‡
Osteoid width, μm	12	11.8	13	14.2†
Placebo				
Formation, %	10	2.3	9	2.3
Resorption, %	6	5.0	5	2.9
Cortical thickness, mm	10	1.0	10	0.7
Trabecular thickness, μm	10	112.1	10	121.3
Osteoid width, μm	10	11.5	9	12.4

*From Kyle RA, Jowsey J, Kelly PJ, et al: Multiple-myeloma bone disease: the comparative effect of sodium fluoride and calcium carbonate or placebo. N Engl J Med 293:1334-1338, 1975. By permission.

Significance of difference from before treatment: †$P<0.01$; ‡$P<0.05$.

four times daily 1 hour after meals and at bedtime, with an identical-appearing placebo regimen. All of the patients received melphalan plus prednisone for 7 days every 6 weeks as basic treatment for their myeloma. At the end of 1 year the studies were repeated and the code was broken. In bone specimens taken before and after fluoride-calcium therapy, microradiography showed that bone formation and trabecular and cortical thickness in the placebo group were unchanged (Table 1). Among the patients who had received fluoride-calcium therapy, however, the cortical and trabecular thickness was significantly increased and resorption was significantly decreased. Increased prominence and thickening of trabeculae were seen in the thoracic and lumbar vertebrae in 6 of the 13 fluoride-calcium patients and in the pelvis of several—but in none of the placebo group. No significant side effects developed. We feel that sodium fluoride and calcium supplementation may be a useful adjunct in treatment for myeloma bone disease.[47]

Anemia.—Anemia is a common finding in myeloma, being noted eventually in almost every case. Transfusion of packed red cells remains the cornerstone of therapy. A hemoglobin concentration of 8 to 10 g/dl is adequate in most cases, unless there is significant coronary-artery disease or cerebrovascular insufficiency. Successful treatment for the primary disease with alkylating agents and corticosteroids may restore erythropoiesis. Androgens may raise the hemoglobin levels in some patients, but the results generally have been disappointing in our experience.

Psychologic Problems.—Any patient with a serious disease such as multiple myeloma needs substantial, continuing emotional support. The approach must be positive. The physician must have confidence in his ability to cope with the patient's problems, and the patient should be able to sense this confidence. Potential benefits of therapy should be emphasized. It reassures the patient to know that some persons survive for 5 years or more while receiving treatment. It is essential that the physicians caring for the myeloma patient have the interest and capacity to deal with incurable disease over a span of months to years with assurance, sympathy, and resourcefulness.

COURSE AND PROGNOSIS

Before the introduction of chemotherapy, median survival was between 3½ and 8½ months; but now median survival is between 2 and 3 years. For *good-risk* patients who *respond* to chemotherapy, a median survival of 53 months has been reported.[10] In another study the median survival was 23 months in 236 patients with myeloma receiving melphalan, prednisone, and procarbazine while median survival was 21 months in 156 patients receiving only melphalan and prednisone.[37] Follow-up information was obtained in 866 of our 869 cases of multiple myeloma diagnosed between 1960 and 1971. The median survival was 20 months. At 1 year the survivorship was 66%, at 3 years 32%, and at 5 years 18% (Fig. 1). Infection and renal insufficiency were the most common causes of death.

The great variation in survival—from a few months to more than 5 years—makes prognosis in individual cases a challenge. Most reports describe the
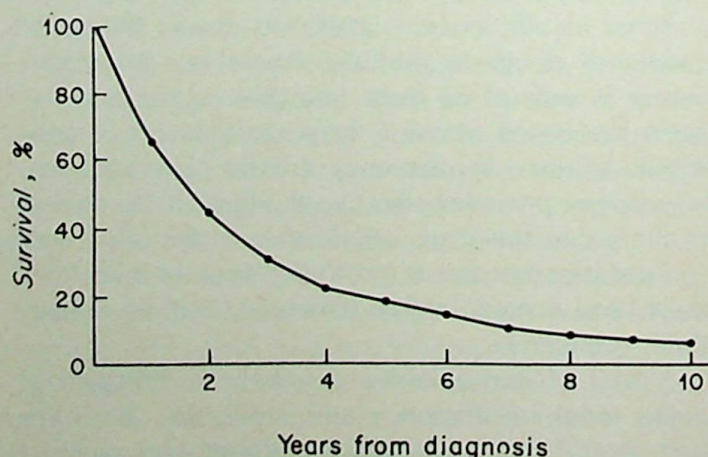
Fig. 1. Multiple myeloma: survival in 869 cases diagnosed between 1960 and 1971. (From Kyle RA, Bayrd ED: The Monoclonal Gammopathies: Multiple Myeloma and Related Plasma-Cell Disorders. Springfield, Illinois, Charles C Thomas, Publisher, 1976. By permission.)

Table 2.—**Multiple Myeloma: Mean Values for Five Variables in 2-Year Survivors and Non-Survivors***

Parameter	Dead (N = 140)	Alive (N = 79)
Serum creatinine, mg/dl	2.9	1.4†
Serum calcium, mg/dl	10.3	9.6†
Hemoglobin, g/dl	10.4	11.2†
Serum albumin, g/dl	2.9	2.9
Serum globulin peak, g/dl	3.6	3.5

*From Kyle RA, Bayrd ED: The Monoclonal Gammopathies: Multiple Myeloma and Related Plasma-Cell Disorders. Springfield, Illinois, Charles C Thomas, Publisher. By permission.
†Significantly different ($P<0.05$).

effects of individual factors but not the simultaneous effect of several factors. Because several prognostic factors usually are involved, multivariate analysis is necessary to assess them adequately. Two hundred nineteen of our cases had all parameters measured and were followed up for at least 2 years. Therefore, we divided our patients with multiple myeloma into two groups—those who died within 2 years and those who survived 2 years or longer—and we applied stepwise linear discriminant analysis to these two groups. The variables included in the analysis were the following: serum creatinine, serum calcium, serum albumin, size of serum globulin peak, hemoglobin level and leukocyte count, palpability of liver or spleen, presence of pain, and age.

Serum creatinine and serum calcium levels were the most significant variables affecting 2-year survival. The hemoglobin level made a modest contribution. Surprisingly, the concentration of monoclonal protein in the serum did not contribute to the prediction of survival or death within 2 years of diagnosis (Table 2) and the values are almost identical in survivors and non-survivors.

The distributions of initial serum creatinine and calcium values from the patients who were surviving at 2 years and from those who were dead at 2 years are seen in Table 3. Calcium values were less than 10 mg/dl and creatinine values less than 2 mg/dl in 46% of those who had died and in 70% of those who survived. Of 25 patients whose initial serum calcium concentration was 12 mg/dl or more, only 2 lived 2 years. Of 28 patients with initial creatinine values of 4 mg/dl or more, only 2 survived 2 years; and of 60 with initial creatinine of 2.0 mg/dl or more, only 6 survived 2 years.

Renal Insufficiency.—All of our patients with multiple myeloma whose *creatinine or urea was measured* initially were divided into two groups on the basis of normality or insufficiency of renal function (creatinine more than 1.2 mg/dl for males and more than 0.9 mg/dl for females, or blood urea more than 50 mg/dl for both males and females). The mean initial serum calcium value from 266 patients with renal insufficiency was significantly greater than that from the 327 patients with normal renal function (10.7 versus 9.5 mg/dl; $P<0.01$) (Table 4). Hypercalcemia (>10.5 mg/dl) was found in 39% of those with renal insufficiency but only 10% of those with normal renal function; and the serum calcium was 12 mg/dl or more in 24% of those with renal insufficiency but only 1% of those with normal renal function. Bence Jones proteinuria was revealed (by heat test) in 58% of patients with renal insufficiency and in 43% of those with normal renal function—a difference of borderline significance.

Table 3.—**Multiple Myeloma: Joint Percentage Distribution of Initial Serum Calcium and Creatinine Values From 79 Patients Who Survived 2 Years and 140 Who Died***

Creatinine, mg/dl	†	Calcium, mg/dl				Total
		<10	10 to 11.9	12 to 13.9	≥14	
<2	S	70	21		1	92
	D	46	12	1	2	61
2 to 3.9	S	1	3	1	1	6
	D	5	6	6	2	19
4 to 5.9	S	1				1
	D	1	4	1		6
6 to 9.9	S					
	D	5	2	1	1	9
≥10	S	1				1
	D	3	1	1		5
Total	S	73	24	1	2	100
	D	60	25	10	5	100

*From Kyle RA, Bayrd ED: The Monoclonal Gammopathies: Multiple Myeloma and Related Plasma-Cell Disorders. Springfield, Illinois, Charles C Thomas, Publisher. By permission.
†S = surviving at 2 years; D = dead at 2 years.

Table 4.—**Multiple Myeloma: Serum Calcium and Creatinine (Within 1 Month of Diagnosis) and Frequency of Bence Jones Proteinuria in Renal Insufficiency and With Normal Renal Function***

Parameters	Renal function	
	Insufficiency	Normal
Serum calcium, mg/dl	(N = 266)	(N = 327)
Mean	10.7	9.5
≥10.5	39%	10%
≥12.0	24%	1%
Bence Jones protein	(N = 299)	(N = 317)
Positive	58%	43%
Urine, monoclonal protein	(N = 80)	(N = 79)
Kappa	54%	63%
Lambda	46%	37%
Serum creatinine, mg/dl	(N = 225)	(N = 182)
Mean	3.5	0.9
>1.3	80%	0%
>2.0	50%	0%

*From Kyle RA, Bayrd ED: The Monoclonal Gammopathies: Multiple Myeloma and Related Plasma-Cell Disorders. Springfield, Illinois, Charles C Thomas, Publisher. By permission.

Of the patients with renal insufficiency (elevated creatinine or urea), 80 had a monoclonal light chain in the urine—kappa in 54% of them and lambda in 46%. Of the patients with normal renal function, 79 had a monoclonal light chain in the urine, which was kappa in 63% of them (Table 4). Hence the presence of a single light chain class was not associated with an excessive incidence of renal insufficiency.

Kappa and Lambda Light Chains in Urine.—Although the 64 patients with kappa monoclonal protein in the urine had a lower serum creatinine than the 55 with lambda, it did not differ significantly. The same is true in amount of urinary globulin excretion (Table 5). Serum creatinine was 2 mg/dl or more in 20% of the patients with kappa monoclonal protein in the urine and in 29% of those with lambda. We found no convincing evidence that monoclonal lambda protein was significantly more nephrotoxic than kappa.

Table 5.—**Multiple Myeloma: Serum Creatinine and Urinary Globulin (Within 1 Month of Diagnosis) in Patients With Monoclonal Light Chains in Urine***

Parameters	Urinary protein	
	Kappa	Lambda
Serum creatinine, mg/dl	(N = 64)	(N = 55)
Mean	1.9	2.3
≥2.0	20%	29%
Urinary globulin, g/24 h	(N = 60)	(N = 53)
Mean	3.8	4.2
≥2.0	45%	55%
≥4	32%	32%
≥6	20%	25%
≥10	10%	11%

*From Kyle RA, Bayrd ED: The Monoclonal Gammopathies: Multiple Myeloma and Related Plasma-Cell Disorders. Springfield, Illinois, Charles C Thomas, Publisher. By permission.

In an attempt to classify myeloma cases by the extent of disease, Durie and Salmon[48] studied 71 patients with multiple myeloma and divided them into three categories based on myeloma cell mass. Those with a large cell mass (>1.2 × $10^{12}/m^2$) had at least one of the following: hemoglobin <8.5 g/dl, serum calcium >12 mg/dl, IgG monoclonal component >7 g/dl, IgA >5 g/dl, Bence Jones proteinuria >12 g/day, or advanced lytic bone lesions. Those with the low cell mass (<0.6 × $10^{12}/m^2$) had all of the following characteristics: hemoglobin >10.0 g/dl, serum calcium normal, serum IgG monoclonal component <5 g/dl or IgA <3 g/dl or Bence Jones proteinuria <4 g/day, and no generalized lytic lesions on a skeletal survey.

Whether the prognosis is related to the type of immunoglobulin abnormality is not clear. We found no significant difference in survival of patients with IgG myeloma and those with IgA myeloma (Fig. 2), or of those with monoclonal light chains of either type in the urine and those without (Table 6). The 3-year survival was 47% among our patients with IgG kappa myeloma and 27% among those with IgA lambda myeloma, but the significance of this difference is borderline because the number of cases is small. Median survival was 31 months with IgG myeloma and 28 months with IgA myeloma. This difference is not significant.
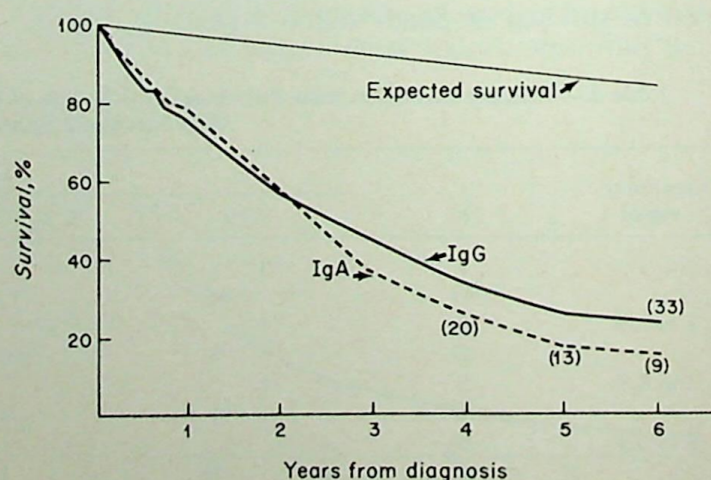
Fig. 2. Multiple myeloma: survival in 308 IgG cases and in 123 IgA cases, with survival normally expected. Difference between myeloma groups was not significant. Parentheses enclose numbers of patients alive and under observation at times plotted. (From Kyle RA, Bayrd ED: The Monoclonal Gammopathies: Multiple Myeloma and Related Plasma-Cell Disorders. Springfield, Illinois, Charles C Thomas, Publisher, 1976. By permission.)

Table 6.—**Multiple Myeloma: Survivorship by Immunoglobulin Type***

Protein	No. of patients	% Surviving at 1 yr	3 yr	5 yr	Median survival
IgG					
Kappa	223	77	47	27	31 mo
Lambda	85	73	39	25	
Total	308†	76	45	26	
IgA					
Kappa	79	84	40	20	28 mo
Lambda	44	70	27	16	
Total	123†	79	36	18	
Urinary light chains (kappa or lambda)‡	95	82	43	18	

*From Kyle RA, Bayrd ED: The Monoclonal Gammopathies: Multiple Myeloma and Related Plasma-Cell Disorders. Springfield, Illinois, Charles C Thomas, Publisher. By permission.
†Patients with IgG and IgA whose light chains were not typed were excluded.
‡Of patients with IgG or IgA in serum.

The rate of response to chemotherapy also appears to affect survival. Hansen and associates[49] reported that those patients whose monoclonal serum protein decreased by 0.6 g/dl or more within 2 months after the start of therapy had a median survival of 13 months, whereas those who responded slowly had a median survival of 62 months. Others have noted that patients who respond rapidly (within 2 months) had shorter median survival as well as a shorter duration of remission than those who responded more slowly.[50] It appears that prognosis is poorer in patients who respond to treatment rapidly, and this most likely is due to greater activity of the disease.

Patients with plasma-cell leukemia or multiple myeloma have a poorer prognosis than those with a solitary lesion, the shorter course reflecting a greater generalized involvement.

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PROGRESS IN COMPUTERIZED TOMOGRAPHY

The Department of Diagnostic Roentgenology, Mayo Clinic, announces a 2½-day continuing education course entitled "Progress in Computerized Tomography." The course will be held on May 25, 26, and 27, 1977, at the Mayo Clinic in Rochester, Minnesota. The program will emphasize computerized tomography of the body but will also include certain aspects of computerized tomography physics and economics and also certain aspects of computerized tomography of the head and breast. The course is approved for AMA Category I credit on an hour-for-hour basis. Registration fee is $200. For further information contact Patrick F. Sheedy II, M.D., CT Course Director, or Robert R. Hattery, M.D., David H. Stephens, M.D., or O. Wayne Houser, M.D., Co-directors, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901.

Development of Radioimmunoassays for Prednisone and Prednisolone

Application to Studies of Hepatic Metabolism of Prednisone

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Radioimmunoassays for measuring prednisone and prednisolone (Δ_1 corticosteroids) in serum have been developed. By using 6,7-^{3}H-Δ_1 corticosteroids as tracer, rabbit antibodies against Δ_1 corticosteroid-21-hemisuccinate in bovine serum albumin, and ammonium sulfate precipitation, the assays detected less than 0.8 ng/ml of Δ_1 corticosteroid and interassay coefficients of variation were less than 8.5%. The specificity of antibodies, tested against all drug metabolites and major endogenous steroids, showed that only 21-glucuronic acid esters of Δ_1 corticosteroids had cross-reactivity of possible clinical importance. Assays were validated by measuring levels in samples of fasting-state sera with or without adding known amounts of prednisolone. Measurements of serum concentrations of both prednisone and prednisolone in normal dogs and in those with hepatic vascular exclusion were made after intravenous administration of prednisone. Although high levels of prednisolone appeared rapidly in normal dogs, only slight amounts were measured in dogs with hepatic vascular exclusion, which emphasizes the importance of the liver in the conversion of prednisone to prednisolone, its active metabolite.

Prednisone is the most commonly used oral synthetic glucocorticosteroid. Like cortisone, prednisone presumably has no anti-inflammatory activity,[1,2] and because conversion to prednisolone (the active metabolite) is complete in states of health,[3,4] doses of prednisone and prednisolone are considered pharmacologically equivalent.[5,6]

Although prednisone has been used for more than 15 years, little information is available about its metabolism in disease or about the relationships between pharmacokinetic measurements and therapeutic effects. Serum prednisolone concentrations have been estimated by using a colorimetric method,[4] competitive protein binding assay,[7] and gas-liquid chromatography.[8] These techniques involve several extraction and chromatographic steps; thus the assays are complex, time-consuming, and not highly sensitive.

The development of radioimmunoassays for prednisolone and prednisone, by Colburn and Buller,[9,10] allows direct measurements of these steroids in serum and now makes it feasible to undertake metabolic and pharmacokinetic studies of prednisone metabolism in health and disease. However, only limited studies of cross-reactivity with steroid were made. We therefore report our development of radioimmunoassays for prednisone and prednisolone. Cross-reactivity testing of antisera was extended to all identified Δ_1 corticosteroid metabolites. A simple method for raising high-titer prednisone antibody is also described and is applied to evaluating the role of the canine liver in converting prednisone to prednisolone.

METHODS

Radioimmunoassays. Reagents: Radioactive Prednisone and Prednisolone.—Tritium-labeled prednisone (6,7-^{3}H-prednisone; specific activity, 40 Ci/mmol) and prednisolone (6,7-^{3}H-prednisolone; specific activity,

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40 Ci/mmol) were generous gifts from The Upjohn Company, Kalamazoo, Michigan, or were obtained from the New England Nuclear Company, Boston, Massachusetts. Purity was greater than 95% when analyzed by thin-layer chromatography (silica gel H; methylene chloride:dioxane:water [2:1:1, lower layer]) and by zonal scanning. A stock solution of radioactive steroid in 0.2 M phosphate buffer at pH 6.5 was stored at 4°C; fresh tracer solutions for use in the radioimmunoassay were prepared weekly by dilution with buffer to a radioactivity concentration of 4,000 cpm/0.25 ml.

Rabbit antisera against either prednisone or prednisolone were also gifts from The Upjohn Company.[9,10] Rabbit antisera against prednisolone were subsequently made by us. For immunogens, prednisolone hemisuccinate was coupled to bovine serum albumin by the mixed anhydride method.[11] Two hundred thirty milligrams of prednisolone-21-hemisuccinate (Steraloids, Inc., Pawling, New York) was dissolved in 5 ml of tetrahydrofuran (dehydrated with Al_2O_3), 0.12 ml of tri-*n*-butylamine (also dehydrated) was added, and the mixture was cooled to 10°C. Later, 0.05 ml of ethyl chloroformate was added (15 minutes reaction time). Meanwhile, 900 mg of bovine serum albumin (Sigma Chemical Company, St. Louis, Missouri) was dissolved by stepwise additions of 40 ml of a mixture of water and tetrahydrofuran (1:1, vol/vol) at 4°C, adjusted to pH 11.0 with 35 mg of NaOH. The mixed anhydride solution was then added by drops during stirring to the bovine serum albumin solution and the reaction was left to proceed overnight at 4°C. After being freeze-dried to remove organic solvents, the powder was reconstituted with 40 ml of water. The prednisolone-bovine serum albumin complex was purified by ultrafiltration (Centriflomembrane, Amicon Company, Lexington, Massachusetts) with three water washes and one acetone wash. The product was lyophilized and stored at room temperature. By using ultraviolet spectroscopy,[11] each molecule of conjugate was calculated to contain 20 steroid moieties.

Immunization.—The immunogen, dissolved in 0.9% NaCl, was emulsified in twice its volume of Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan). One milliliter of this mixture (containing 50 μg of steroid) was injected intracutaneously at 10 sites into the skin of New Zealand white rabbits. After 4 weeks, a booster injection (50 μg of steroid in 0.5 ml of 0.9% NaCl) was given intramuscularly and, 10 days later, 30 ml of blood was taken from an ear vein. Serum was separated after centrifugation and stored at −70°C. Stock solutions of antisera (1:10 dilution with buffer) were stored at −20°C. About twice a month, final dilutions of antisera were made for use in the radioimmunoassay and comprised prednisone 1:150, prednisolone I (Upjohn) 1:400, and prednisolone II (Mayo Clinic) 1:2,000. All were kept frozen at −20°C and were thawed immediately before use.

Standards.—Prednisone, prednisolone, tetrahydrocortisol, tetrahydrocortisone, 1,4-androstadien-3, 11,17-trione, 5β-androstan-3-11-diol-17-one, 5β-androstan-3-ol-11,17-dione, cortisol, cortisone, testosterone, progesterone, androstenedione, and dexamethasone were obtained from Steraloids, Inc. Prednisone glucuronide and prednisolone glucuronide were kindly synthesized by Dr. Vernon R. Mattox, and 20-hydroxyprednisolone was synthesized from prednisolone with sodium borohydride.[12] 20-Hydroxyprednisone was kindly donated by the Schering Company, Bloomfield, New Jersey, and by Merck Sharp & Dohme, Rahway, New Jersey.

Stock solutions of steroids were prepared by dissolving 0.1 mg in 95% ethanol to yield 50 μg/ml. Using charcoal-extracted human serum, diluted 1:50 with 0.2 M phosphate buffer at pH 6.5 (CEHS 1:50), standards containing 0.1 ng to 10.000 ng per milliliter were made, kept frozen at −20°C, and thawed immediately before use. The stock solutions of prednisone, prednisolone, and cortisol were checked by a colorimetric method using the Porter-Silber reagent (through the courtesy of Dr. Nai-Siang Jiang).

Procedure.—Standard or unknown (0.1 ml, diluted 1:50 with buffer) material was first mixed with 0.25 ml of tracer solution (0.2 M phosphate buffer, pH 6.5, containing 4,000 cpm of ^3H as a tracer) by using an automatic pipet (Micromedic, Medical Laboratory Automation, Philadelphia, Pennsylvania) in 12- by 75-mm glass tubes (Scientific Products, McGraw Park, Illinois); 0.1 ml of antibody and 0.25 ml of buffer (0.2 M phosphate buffer, pH 6.5) were added subsequently to make a final volume of 0.7 ml. Incubation was carried out at room temperature for 1 hour.

Separation of free and bound tracer was accomplished by precipitating bound tracer with the addition of 0.7 ml of a saturated ammonium sulfate solution (80%, wt/vol), then placing the tubes at 4°C in a cold room for 1 hour, and later centrifuging at 1,200 × g at 4°C for 25 minutes. The supernatant containing free tracer was decanted into a scintillation vial and 10 ml of a scintillation solution (toluene, 1 gal., + butyl PBD [Beckman Primary Fluor, Irving, CA], 28.5 gr) was added. After 18 hours in darkness, "free" radioactivity was measured in a Beckman LS-
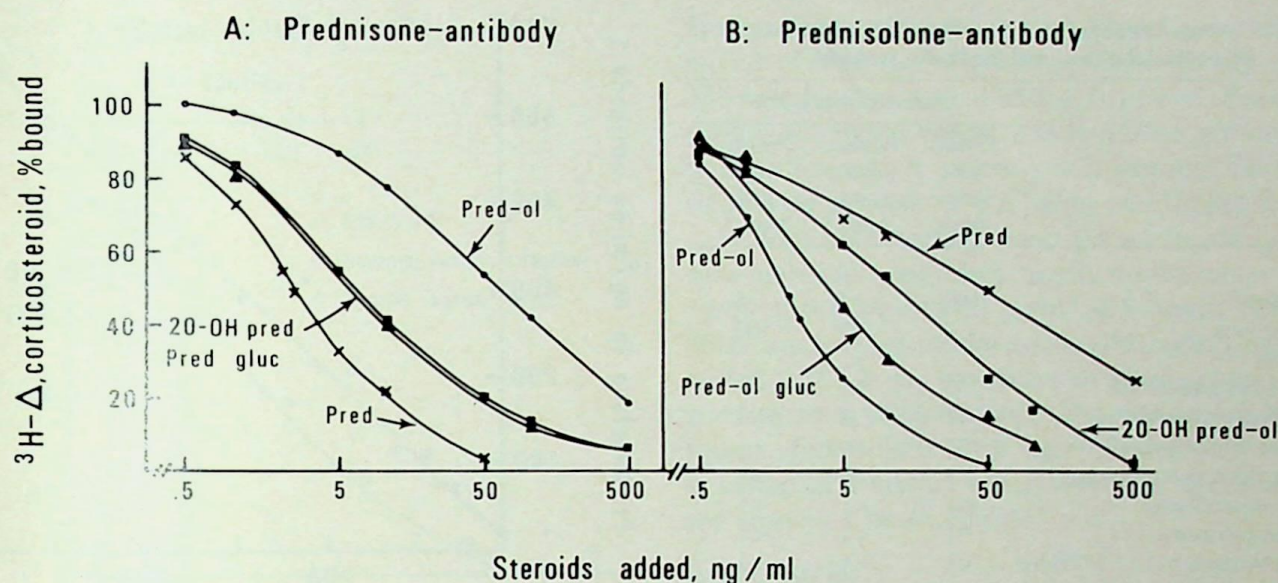
Fig. 1. Sensitivity and specificity of assays. Note appreciable displacement of ^{3}H-prednisone by 0.8 ng/ml of prednisone (x) and by 0.6 ng/ml of prednisolone (•), respectively. In prednisone assay, 20-hydroxyprednisone (▪) and prednisone glucuronide (▲) show cross-reactivity; in prednisolone assay, 20-hydroxyprednisolone and prednisolone glucuronide show cross-reactivity.

250 liquid scintillation counter with an efficiency of about 40%, the error of counting being ±2%.

Calculation of results was based on the inclusion of two sets of blanks, each comprising both a diluent blank (containing 0.1 ml of CEHS 1:50, 0.25 ml of tracer, and 0.35 ml of buffer) and an antibody blank (containing 0.1 ml of CEHS 1:50, 0.25 ml of tracer, 0.1 ml of antibody, and 0.25 ml of buffer). Bound radioactivity (in percent) was calculated by subtracting the "percent free" (= cpm in supernatant of standard or unknown) from the 100% free (= cpm in supernatant of diluent blank). Bound radioactivity of the antibody blank (maximal binding) was normalized to 100%, other results being expressed as a percentage of this. With a displacement (standard) curve, results of serum analyses were calculated in nanograms per milliliter.

Validation.—Fasting-state sera, presumed to be devoid of prednisone or prednisolone, were analyzed from 3 normal dogs, 3 dogs with hepatic vascular exclusion, 20 normal volunteers, and 32 patients with chronic active liver disease (CALD), with and without previous prednisone therapy. Those who had been taking prednisone had discontinued the medication at least 48 hours previously. In addition, known amounts of prednisolone (100 to 600 ng/ml) were added to fasting-state pooled normal serum for measurements of recovery. Also, serial dilutions of two human sera containing about 300 ng/ml of prednisolone were assayed to determine independence of dilution.

Preliminary Application.—Three normal dogs and three dogs with hepatic vascular exclusion were given an intravenous injection of prednisone prepared in a concentration of 3 mg/ml of 50% ethanol; 0.2 mg/kg was given over 5 minutes after induction of anesthesia (0.5 mg of atropine, 5 ml of Innovar, and 60 to 300 mg of pentobarbital, intravenously). Anesthesia was maintained with pentobarbital. The dogs with hepatic vascular exclusion had undergone a portacaval shunt, ligation of the hepatic artery, and cleavage of all other liver attachments except hepatic and caval veins. All dogs received 5% glucose with 0.9% NaCl intravenously to maintain urine outputs at more than 0.5 ml/min. Blood pressure and temperature were monitored and were normal during the 5-hour study period. Blood samples were taken at 0, 0.1, 0.3, 0.5, 1, 2, 3, 4, and 5 hours after prednisone was injected. After clotting, serum was separated by centrifugation and was stored at −70°C until analysis.

RESULTS

Sensitivity and Specificity of Assays.—The final assay conditions regarding buffer pH, buffer-ionic strength, and time and temperature of incubation were shown to be optimal for all three antibodies used. The dilution of antiserum bound about 45% of the tracer dose; the final dilution was 1:1,050 for the prednisone antiserum, 1:2,800 for the prednisolone antiserum I, and 1:14,000 for the prednisolone antiserum II. With these amounts of antisera, 0.8 ng/ml (40 ng/ml of serum, diluted 1:50) of prednisone and 0.6 ng/ml

Table 1.—**Cross-Reactivity of Prednisone, Prednisolone, Their Metabolites, and Synthetic Steroids**

	Cross-reactivity, %*		
	Prednisone antibody	Prednisolone antibody I	Prednisolone antibody II
Drugs			
Prednisone	100	3	4
Prednisolone	4	100	100
Metabolites			
20-Hydroxyprednisone	37	<1	<1
20-Hydroxyprednisolone	<1	6	14
Prednisone glucuronide	35	4	<1
Prednisolone glucuronide	3	65	46
Tetrahydrocortisone	<1	<1	<1
Tetrahydrocortisol	<1	<1	<1
1,4-Androstadien-3,11,17-trione	<1	<1	<1
5β-Androstan-3,11-diol-17-one	<1	<1	<1
5β-Androstan-3-ol-11,17-dione	<1	<1	<1
Endogenous steroids			
Cortisone	32	<1	<1
Cortisol	<1	18	15
Testosterone	<1	<1	<1
Progesterone	<1	<1	<1
Androstenedione	<1	<1	<1
Synthetic steroid			
Dexamethasone	<1	4	5

$$\text{* \% Cross-reactivity of compound X} = \frac{\text{ng/ml } \Delta_1 \text{ corticosteroids causing 50\% displacement of label}}{\text{ng/ml compound X causing 50\% displacement of label}} \times 100.$$

(30 ng/ml of serum, diluted 1:50) of prednisolone caused detectable replacements of tracer and therefore defined the limits of sensitivity of our systems.

Cross-reactivity of possible significance was found for cortisone, cortisol, 20-hydroxy derivatives of prednisone and prednisolone, and the 21-substituted esters (such as glucuronides) of prednisone and prednisolone (Fig. 1). The relative amounts of pure steroids required to displace 50% of bound tracer are given in Table 1. Cross-reactivity of endogenous corticosteroids and 20-hydroxy derivatives is approximately 15%; for 21-glucuronic esters, it is larger—up to 50%. Other endogenous steroids and metabolites of Δ_1 corticosteroids did not show cross-reactivity.

Specificities of the two prednisolone antibodies were comparable and a good correlation (r = 0.97) was obtained when 20 sera were assayed using both (Fig. 2).

Validation.—Immunoreactive prednisone and prednisolone values were not detected in fasting-state sera from normal dogs and dogs with hepatic
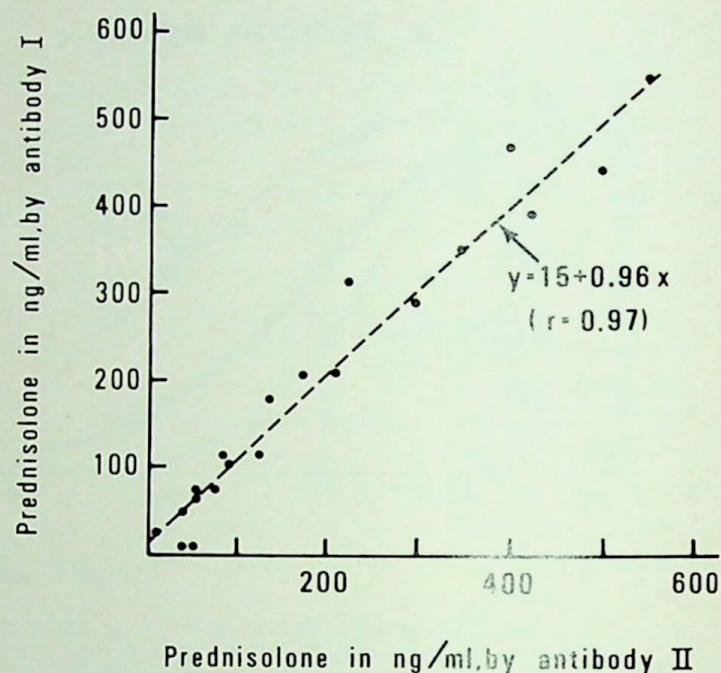
Fig. 2. Correlation among prednisolone values in 20 human sera by using prednisolone antibody I (Upjohn) and prednisolone antibody II (Mayo Clinic). Line represents regression line.

vascular exclusion. In healthy humans and patients with CALD, presumably because of cross-reacting cortisol, very small amounts of prednisolone were detected (Fig. 3). Recovery of added prednisolone, 100 to 500 ng/ml of fasting human pooled sera, in 10 studies was 103 ± 3% (mean ± 1 SE). Displacement curves with serial dilutions of human sera containing prednisolone resembled the standard displacement curve (Fig. 4).

The interassay variation, determined from 35 consecutive measurements of two internal standard sera
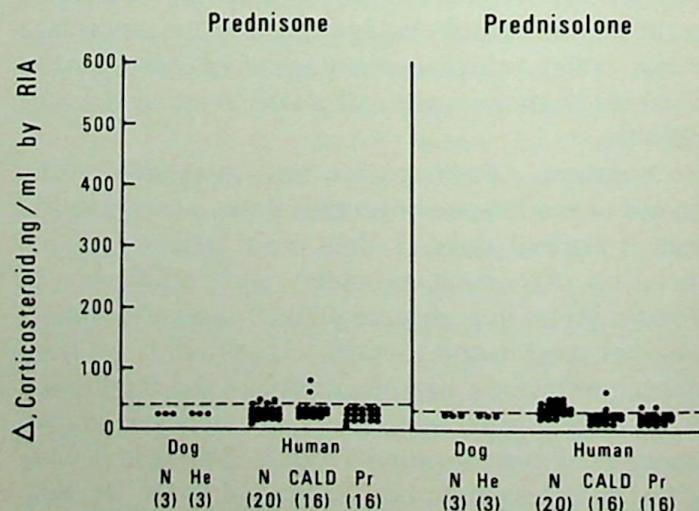
Fig. 3. Δ_1 Corticosteroid values in fasting-state serum from normal dogs (*N*) and dogs with hepatic vascular exclusion (*He*), normal human volunteers (*N*), and patients with chronic active liver disease with (*Pr*) and without (*CALD*) prednisone therapy.
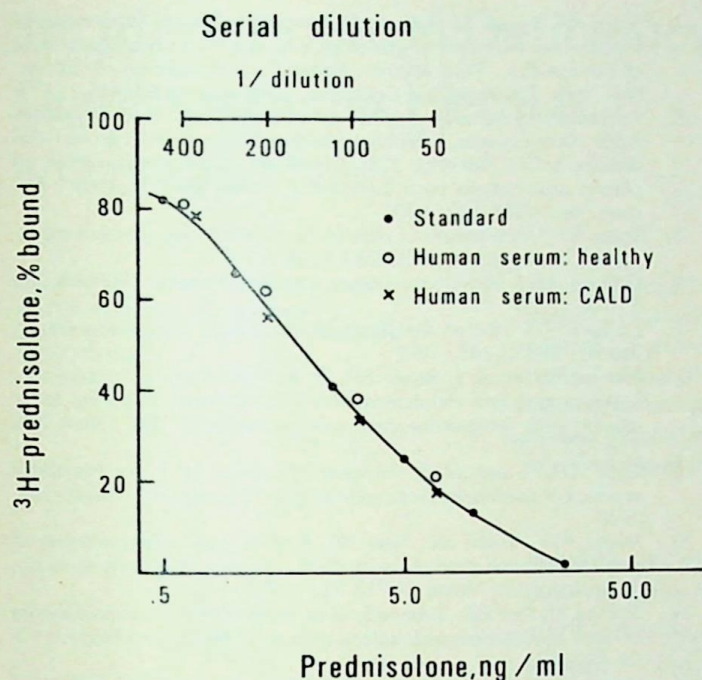
Fig. 4. Similarity is shown between serial dilutions of prednisolone containing human serum and displacement curve made from standard solutions of prednisolone.

with about 20 and 40% binding, respectively, was 5.9 to 8.5% for prednisolone and 5.8 to 8.4% for prednisone.

Preliminary Application.—After intravenous injection of prednisone in normal dogs, serum prednisone levels rose to 407 ng/ml (range, 190 to 650) at 0.1 hour; after 5 hours only minimal amounts (mean, 47 ng/ml; range, 30 to 75) were detectable.
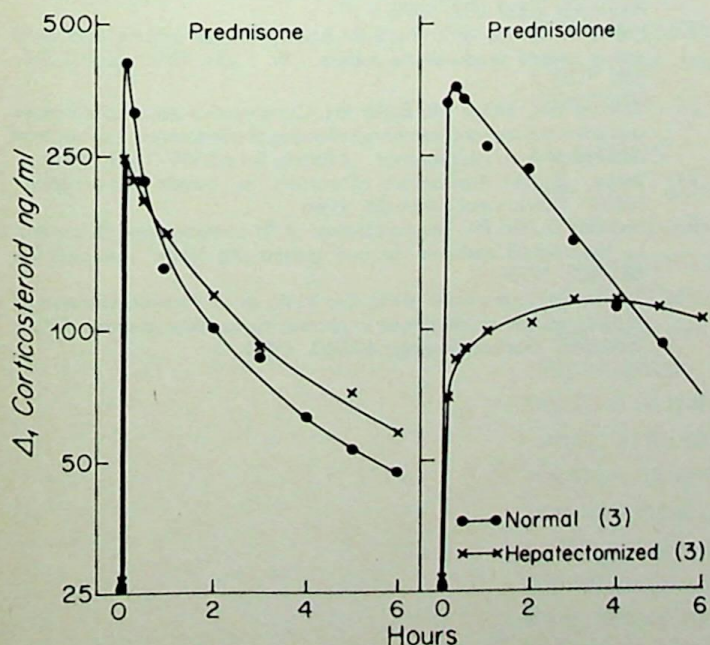
Fig. 5. Δ_1 Corticosteroid values after intravenous injection of prednisone into normal dogs (•) and dogs with hepatic vascular exclusion (x). Each point represents mean of three measurements.

Prednisolone appeared in the blood soon after injection of prednisone. Mean serum prednisolone was 335 ng/ml (range, 120 to 550) at 0.1 hour. Peak levels (mean, 360 ng/ml; range, 158 to 550) occurred at 0.3 hour, after which serum prednisolone decreased steadily to a mean of 92 ng/ml at 5 hours (Fig. 5).

In dogs with hepatic vascular exclusion, prednisone levels decreased less rapidly from a mean of 255 ng/ml at 0.1 hour to 82 ng/ml at 5 hours. Strikingly small amounts of prednisolone (70 ng/ml) were detected at 0.1 hour, indicative of slow conversion of prednisone to prednisolone. The mean peak level of serum prednisolone (116 ng/ml; range, 98 to 145) occurred at 3 hours. There was little decrease over the ensuing 2 hours (Fig. 5).

DISCUSSION

Our results indicate that radioimmunoassays for prednisone and prednisolone that are accurate for clinical purposes can be developed. The antibodies have considerable specificity for the hydroxy or keto group at the 11-position and the double bond at the 1,4-position. These findings are in accord with those of Colburn and Buller[9,10] except for the larger cross-reactivity between prednisone and 20-hydroxyprednisone found by us.

Previous reports on the radioimmunoassay of prednisolone[9,10,13,14] do not include cross-reactivity data for its metabolites[12,15,16,17] other than for 20-hydroxyprednisolone. In our study, cross-reactivity of appreciable significance was found for both the 20-hydroxy derivatives and the glucuronides of prednisone and prednisolone; other metabolites did not interfere. Interference of 20-hydroxy derivatives of prednisone and prednisolone is unlikely to be quantitatively important, if our analogy with 20-hydroxycortisol is valid, because of the more rapid elimination of the 20-hydroxy derivatives.[18] By contrast, glucuronides of corticosteroids can be less readily discounted because some glucuronide may be present 4 hours after injection of prednisolone.[16] Several investigators,[7,13,17,19,20] recognizing this problem, have performed extraction and chromatography before quantitative analysis. Although cumbersome and time-consuming, this procedure may not increase accuracy because of the large coefficient of variation of 17%.[13] Future alternatives may include performing radioimmunoassays in methylene chloride or ethyl acetate serum extracts, which do not contain conjugated corticosteroids, or using antibodies with increased specificity for the side chain.

Cross-reactivity for cortisol is the most likely cause of slightly elevated prednisolone concentrations in

fasting-state serum from normal volunteers. However, in patients ingesting prednisone, cortisol values are low throughout the 24-hour day.[21,22] This would explain the absence of such cross-reactivity in fasting sera from patients treated with prednisone. In such individuals, more valid estimation of serum prednisolone values can therefore be made.

The comparative serum levels of prednisone and prednisolone resulting from their interconversion have been investigated by Colburn and associates[23] after administration of prednisone or prednisolone to dogs. They found that the combined prednisone and prednisolone areas under the serum concentration-time curves were similar, whichever drug was given.[23] Conversion of prednisone to prednisolone may be impaired in liver failure because 11-hydroxylation is considered primarily a hepatic process.[24] In our experimental canine model with maximal impairment of hepatic function (circulatory exclusion of the liver), there was a striking reduction in prednisolone concentrations after intravenous administration of prednisone, and the small but sustained appearance of prednisolone was probably due to its production in extrahepatic tissues.[24,25] However, in patients with severe CALD who were studied by a similar method, the relatively minor changes in prednisone metabolism detected by us[26] suggest that severely impaired conversion may occur only with very extensive hepatic damage.

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Incidence of Iron Deficiency Anemia in Patients With Large Diaphragmatic Hernia

A Controlled Study

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The incidence of anemia in 259 patients with a diaphragmatic hernia large enough to be seen on a routine chest roentgenogram was compared with that in 259 age- and sex-matched controls. Eighteen patients with diaphragmatic hernia were anemic, compared to one control subject ($P<0.001$). In thirteen patients with diaphragmatic hernia and in one control the anemia was proven to be caused by iron deficiency. The findings provide additional evidence that a large diaphragmatic hernia can cause anemia secondary to chronic gastrointestinal blood loss, which is usually not the result of reflux esophagitis.

Anemia caused by gastrointestinal blood loss has often been observed in patients who have large diaphragmatic hiatus hernias.[1-5] In a review of eight series including 1,305 patients with diaphragmatic hernia, 20% were anemic.[6] However, none of these reports included controls without diaghragmatic hernia, which is a common condition, especially in older persons. For example, Wolf and associates[7] found a medium or large diaphragmatic hernia in 19% of patients 60 years of age or more undergoing routine barium meal examination. Most anemic patients with diaphragmatic hernia do not have endoscopic evidence of esophagitis.[5,6,8] Winans[9] therefore questioned whether anemia really is commoner in patients with diaphragmatic hernia, concluding that a diaphragmatic hernia is not in itself an adequate explanation for anemia. To try to answer this question, the present work was undertaken. The aim was to compare the incidence of anemia in patients with large diaphragmatic hernias and matched controls. Unlike previous studies, patients were selected by a method that did not take gastrointestinal symptoms into account, so as to encompass a wider population of patients with diaphragmatic hernia than those usually seen in the Division of Gastroenterology.

METHODS

The study extended from January 1973 to December 1975. All Mayo Clinic outpatients whose routine chest roentgenograms (Fig. 1) showed a diaphragmatic hernia but no other abnormalities were included. A control patient of the same sex and within 5 years of the same age, whose routine chest roentgenogram was normal, was matched with each patient with diaphragmatic hernia. The controls were selected so as to include proportions of patients newly registered and of those seen in previous years similar to those in the group with diaphragmatic hernia. The routine chest roentgenogram request form contains no clinical information and none was available at the time the patients and the controls were selected.

Advantage was taken of the fact that patients having a routine chest roentgenogram almost always have a routine blood count at the same time, before the result of the chest roentgenogram is available. Only one patient, a control, had to be replaced in the series because no blood count was done. The charts of these patients were examined at the conclusion of
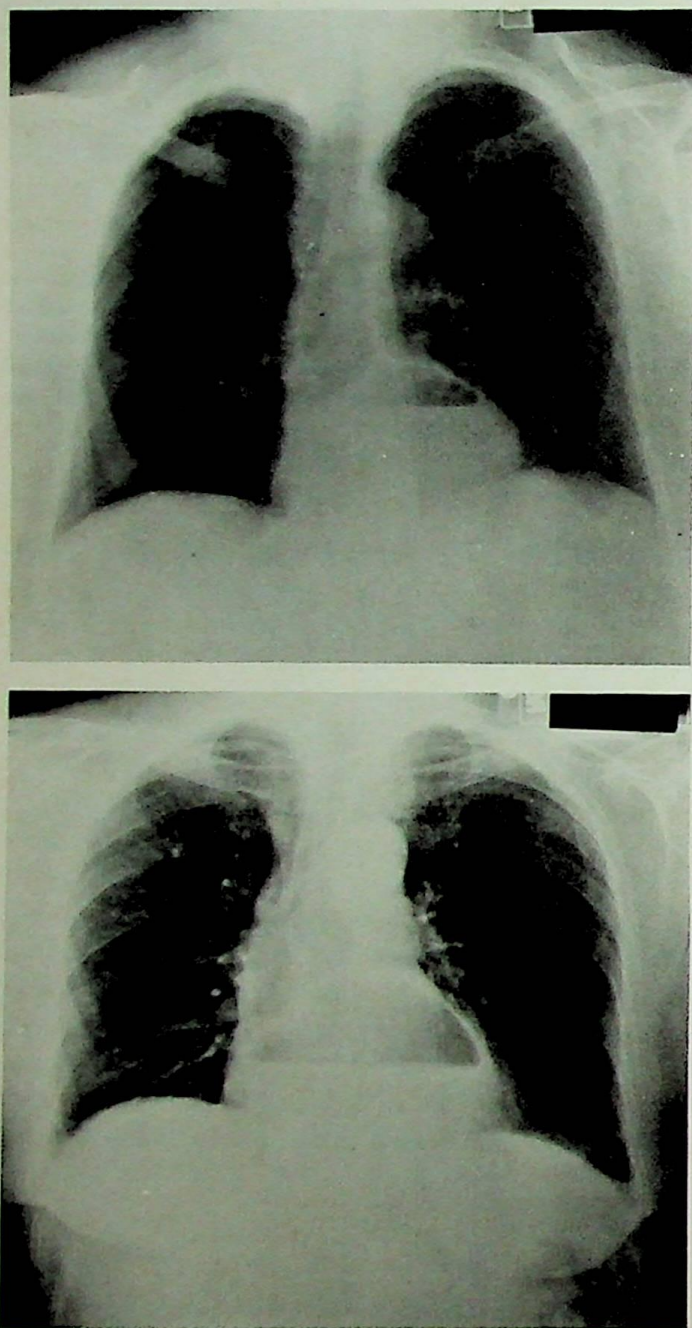
Fig. 1. Chest roentgenograms showing diaphragmatic hernias in two patients with iron deficiency anemia.

the study. When anemia was present, further information about it was taken from the chart.

Anemia was defined as existing when the hemoglobin level was less than 10 g/dl in women or 12 g/dl in men. Iron deficiency was considered proven if the serum iron-binding capacity was 5% saturated or less or if saturation was 6 to 15% in the presence of reduced stores of bone marrow iron or documented correction of anemia with iron therapy.[10] The red cell morphology also had to be consistent with iron deficiency. One patient with diaphragmatic hernia and two controls, all of whom had anemia of other recognized cause (leukemia, thalassemia, and Whipple's disease), were excluded. The significance of controlled observations was calculated by the chi-square test.

RESULTS

Eighteen of 259 patients with diaphragmatic hernia (6.9%) were anemic at the time of study compared with 1 of 259 controls (0.4%), $P<0.001$. Mean hemoglobin level in the anemic patients with diaphragmatic hernia was 8.7 g/dl. In 13 anemic patients with diaphragmatic hernia and 1 control, the anemia was proven to be caused by iron deficiency. Two other anemic patients with diaphragmatic hernia probably had iron deficiency with iron saturations of 6% and 8%, and three anemia patients were not further investigated.

Iron deficiency anemia may be intermittent. Nine more patients with diaphragmatic hernia with normal hemoglobin levels at the time of study had documented iron deficiency anemia on a previous clinic visit, compared with no documented iron deficiency anemia in the controls. Twenty-one (8.1%) of the 259 patients with diaphragmatic hernia were taking iron medication at the time of study, compared with 4 (1.5%) controls.

The diagnosis of diaphragmatic hernia by chest roentgenogram was generally correct. Upper gastrointestinal roentgenograms confirmed the presence of diaphragmatic hernia in 173 (66.8%) of 259 cases and showed no diaphragmatic hernia in 2 (0.8%) patients (neither of whom were anemic); roentgenograms were not done in the remaining 84 (32.4%) cases.

The exact origin of blood loss in the iron deficient patients with diaphragmatic hernia could rarely be determined. Three had a history suggesting gastroesophageal reflux, six had nonspecific symptoms such as chest discomfort or fullness after large meals, and four had no gastrointestinal complaints. Only one had had melena within the previous month. Upper gastrointestinal roentgenograms showed only a paraesophageal or sliding diaphragmatic hernia in 10 anemic patients, 1 of these having the entire stomach above the diaphragm. Besides the diaphragmatic hernia, roentgenograms showed a postcricoid web, a pharyngoesophageal diverticulum, and a duodenal bulb deformity in one patient each. On fiberoptic upper gastrointestinal endoscopy, two esophageal ulcers were found in a patient with a Barrett esophagus, mild esophagitis was found in one patient, and there was no mucosal abnormality in three. Colon roentgenograms in 11 patients, small bowel roent-

genograms in 4, and surgical exploration in 3 failed to reveal any other source of chronic blood loss.

Retrospectively, chart review produced evidence of esophageal reflux symptoms in 33 of 259 patients with diaphragmatic hernia but in only 16 controls; but this difference was thought to be partly caused by a more persistent search for symptoms once a diaphragmatic hernia had been found.

The patients in this study were generally attending the clinic for routine physical examinations or non-urgent medical disorders. Their average age was 69 years (range, 32 to 89) and 74% were women. The most frequent diagnoses, other than diaphragmatic hernia, included degenerative arthritis, hypertension, cataracts, prostatic hypertrophy, tension headaches, and obesity.

DISCUSSION

This study showed that anemia, usually of iron deficiency type, was 18 times commoner in patients with large diaphragmatic hernias than in control subjects. This association suggests that diaphragmatic hernia causes anemia. Chronic gastrointestional blood loss was demonstrated in anemic patients with diaphragmatic hernia by Holt and associates,[5] who showed a mean daily blood loss of 15 ml, compared with 3 ml/day in those without anemia. Mean absorption of an oral iron dose was 39% in their anemic patients with diaphragmatic hernia and 8% in those without anemia, so impaired iron absorption was not a contributory factor.

The cause of gastrointestinal blood loss in patients with diaphragmatic hernia is often assumed to be reflux esophagitis but this is probably incorrect. Most anemic patients in the present series had no esophageal reflux symptoms, and most anemic patients with diaphragmatic hernia had no endoscopic evidence of esophagitis.[5,6,8] Conversely, anemia resulting from chronic blood loss is unusual in patients who have gross esophagitis.[6] The suggestion of Windsor and Collis[6] that bleeding occurs from mechanical trauma to the gastric mucosa within the hernial sac seems reasonable but is rarely substantiated by endoscopic demonstration of a lesion of the gastric mucosa.

Further evidence that the hernia causes the anemia is the response to surgery. Diaphragmatic hernia repair usually prevents recurrence of the anemia[6,8,11] and reduces occult blood loss.[5] Many of these elderly patients are poor surgical candidates but the anemia can also be corrected by oral iron therapy.[5]

The finding of a large diaphragmatic hernia in a patient who has iron deficiency anemia indicates that the cause of the anemia has probably been found and that a further extensive search for another cause of blood loss is unlikely to be rewarding. Roentgenographic examination of the lower gastrointestinal tract would be reasonable but exploratory laparotomy generally is unnecessary.

The findings in the present study cannot be extrapolated to patients who have small hernias, which, clinical experience suggests, are rarely associated with anemia caused by chronic blood loss.

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Hepatic Lipids in Reye-Johnson Syndrome and in Acute Encephalopathy Without Fatty Liver

The relationship between Reye-Johnson syndrome and acute encephalopathy without fatty liver was investigated by comparing the lipid composition of liver samples obtained from five patients with Reye-Johnson syndrome, two patients with acute encephalopathy, and five controls. The mean total hepatic triglyceride concentration was increased nearly sevenfold in Reye-Johnson syndrome and slightly decreased in acute encephalopathy when compared with the mean control value. The mean total hepatic free fatty acid concentration was increased nearly threefold in acute encephalopathy when compared with the mean value in Reye-Johnson syndrome. Total phospholipid content was decreased in the liver in Reye-Johnson syndrome, and this difference was caused mainly by a diminution of the hepatic lecithin fraction. The ratio of palmitic acid to oleic acid in hepatic free fatty acids was 2.5 in Reye-Johnson syndrome, 0.7 in acute encephalopathy, and 0.8 in controls. These results suggest that, despite clinical similarities and laboratory evidence of hepatic dysfunction in both Reye-Johnson syndrome and acute encephalopathy, different pathogenic mechanisms may be responsible for the liver abnormalities found in the two syndromes.

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The association of acute encephalopathy with fatty changes in the viscera was described as a distinct syndrome of childhood simultaneously in 1963 by Reye, Morgan, and Baral[1] in Australia and by Johnson, Scurletis, and Carroll[2] in the United States. Although the fatty changes have been found in kidney, pancreas, spleen, heart, and skeletal muscle, the most striking involvement usually occurs in the liver. Reye and his associates[1] described uniform and complete fatty change throughout the liver and noted that ". . .every cell in every lobule is packed with fatty droplets." Since then, a grossly fatty liver has been considered an essential pathologic feature in Reye-Johnson syndrome. However, in 1972 Glasgow and associates[3] reported on two patients who had acute encephalopathy and laboratory evidence of hepatic dysfunction compatible with Reye-Johnson syndrome but who did not have severe fatty changes in the liver. Histochemical studies revealed minimal fatty deposits with preferential distribution among hepatocytes at the lobular periphery. Glasgow and associates suggested that these cases represented examples of a mild stage in the development of fatty liver in Reye-Johnson syndrome but provided no information on the lipid composition of liver to support this contention. Furthermore, reports of quantitative analyses of hepatic lipids in Reye-Johnson syndrome have been few and lack agreement. A pronounced rise in trigylcerides was the only hepatic lipid abnormality found by Norman and his associates[4] in three patients with Reye-Johnson syndrome. Bourgeois and associates[5] noted a marked increase in both triglycerides and free fatty acids in livers from patients with Reye-Johnson syndrome but gave no quantitative results. Pollack and associates[6] analyzed liver samples from five patients with Reye-Johnson syndrome and found increased levels of triglycerides and decreased levels of phospholipids; however, normal control values were not available from the same laboratory.

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Table 1.—**Clinicopathologic and Laboratory Data From Seven Patients With Reye-Johnson Syndrome and From Two Patients With Acute Encephalopathy Without Fatty Liver**

					Cerebrospinal fluid				
Patients*	Age, yr	Prodrome	SGOT (U/liter)	Blood NH_3 (μg/dl)	Cells† (per mm³)	Protein (mg/dl)	Sugar (mg/dl)	Fatty liver	Comments
1	15	URI‡	133	410	. . .	. . .	. . .	Yes	Exchange transfusion
2	9	URI	1,292	88	. . .	. . .	. . .	Yes	CPK,‡ 34,000
3	8	Varicella	279	162	4L, 1P	. . .	55	Yes	Exchange transfusion
4	10	URI	224	217	3L, 1P	12	80	Yes	Exchange transfusion
5	9	URI	834	690	0L, 0P	41	38	Yes	Peritoneal dialysis; blood sugar, 26
6	3	Varicella	674	101	. . .	. . .	. . .	Yes	Peritoneal dialysis; blood sugar, 43; CPK, 3,700
7	14	Vomiting	1,638	137	3L, 0P	12	73	. . .	Exchange transfusion; survived
8	14	URI, abdominal pain	2,036	297	2L, 0P	31	80	No	
9	1	Gastroenteritis	3,213	151	2L, 0P	22	81	No	*Shigella flexnerii* cultured from stool

*All patients were in coma; all but no. 7 (who survived) had cerebral edema.
†L = lymphocytes; P = polymorphonuclear leukocytes.
‡URI = upper respiratory infection; CPK = creatine phosphokinase.

We report here the results of quantitative analyses of hepatic lipids in five patients with Reye-Johnson syndrome, in two patients with acute encephalopathy without severe fatty changes in liver, and in five controls. Differences were found in concentrations of triglycerides, phospholipids, and free fatty acids and in fatty acid profiles of the triglyceride and free fatty acid fractions. These results provide evidence against the assumption that acute encephalopathy represents a forme fruste of Reye-Johnson syndrome and suggest that different pathogenic mechanisms may be responsible for the liver abnormalities observed in the two syndromes.

MATERIALS AND METHODS

Selection of Patients.—A presumptive diagnosis of Reye-Johnson syndrome was made on nine children seen at the Mayo Clinic during a 15-month period from January 1974 through March 1975. Criteria used for diagnosis included: (1) acute encephalopathy characterized by rapid deterioration of the level of consciousness and often preceded by a prodromal illness, (2) lack of cerebrospinal fluid pleocytosis, and (3) laboratory evidence of hepatic dysfunction, including elevated blood ammonia and SGOT values. One patient survived and eight patients died, despite treatment that included peritoneal dialysis and exchange transfusions. At autopsy, cerebral edema, absence of central nervous system infection, and severe fatty changes in the liver confirmed the diagnosis of Reye-Johnson syndrome in six of the eight patients. In the remaining two patients, the liver appeared grossly normal at postmortem examination. Clinicopathologic and laboratory data from the nine patients are shown in Table 1. The two patients with acute encephalopathy and absence of fatty liver are described in more detail.

Case 1.—A 14-year-old boy was admitted to the hospital in coma. He had been well until 5 days before admission, when he developed fever, cough, headache, and abdominal pain. His father and one sibling were reportedly also sick with "the flu." Four days before admission, the patient vomited but, on the following day, he improved and was able to attend school. On returning home he complained of abdominal pain, and fever recurred. Vomiting became protracted, and he appeared lethargic. Two days before admission he became delirious.

On admission, he was hyperpneic and exhibited decerebrate posturing with bilateral extensor responses to plantar stimulation. Lumbar puncture yielded cerebrospinal fluid containing 2 lymphocytes per mm³, a protein concentration of 31 mg/dl, and a sugar concentration of 80 mg/dl. The SGOT value was 2,036 U/liter and the blood ammonia value was 297 μg/dl. He developed respiratory arrest and his pupils became unreactive to light. He died 9 days after his illness began. At autopsy, there was cerebral edema and herniation of the cerebellar tonsils. The liver appeared normal on gross examination. Microscopic sections of the liver stained with Oil Red O showed mild infiltration with small neutral lipid droplets that were distributed preferentially among hepatocytes at the lobular periphery.

Case 2.—A 1-year-old boy was admitted to the hospital in coma. He had been well until 6 days before admission, when he developed vomiting and diarrhea. Four days later he was admitted to a local hospital, where he was found to be febrile and lethargic and had focal twitching of the left eye and left foot. His condition deteriorated rapidly and he became unresponsive to painful stimulation. Respirations were described as Cheyne-Stokes type and his pupils became dilated and fixed. Cerebrospinal fluid examination
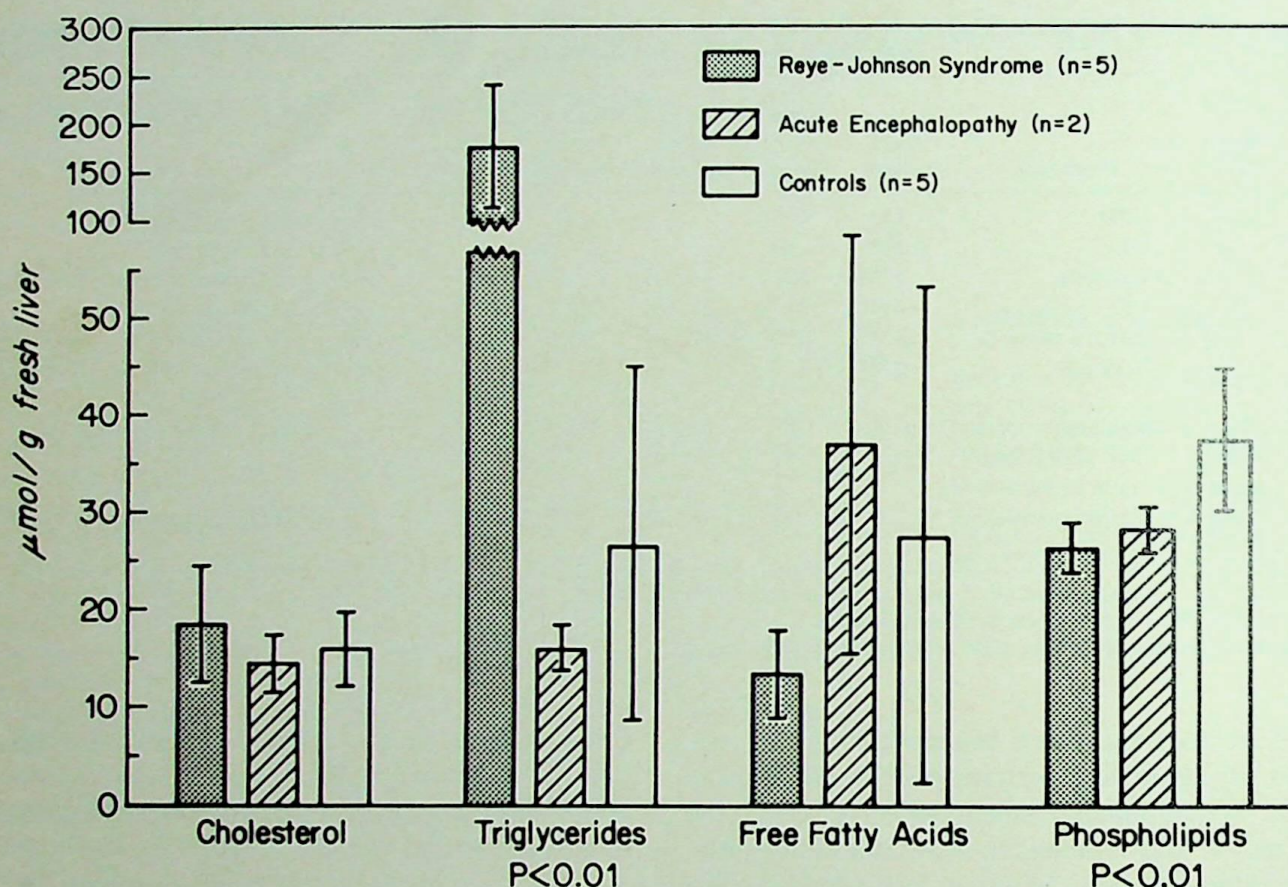
Fig. 1. Hepatic lipid profiles in Reye-Johnson syndrome, in acute encephalopathy, and in controls.

showed 2 lymphocytes per mm^3, a protein concentration of 22 mg/dl, and a sugar concentration of 81 mg/dl. The SGOT value was 3,213 U/liter and the blood ammonia value was 151 μg/dl. He died 1 hour after he was transferred to Mayo Clinic. At autopsy, there was cerebral edema and herniation of the cerebellar tonsils. The liver was grossly normal but microscopic examination of sections stained with Oil Red O demonstrated moderately severe fatty changes predominating at the lobular periphery. *Shigella flexnerii* was cultured from stools.

Liver samples from seven of the nine patients were obtained at autopsy and stored at −12°C for subsequent lipid analyses. Tissue was not available from two patients with Reye-Johnson syndrome (patients 1 and 7, Table 1). Postmortem liver samples were also obtained from two adolescents and two adults who died of cardiac arrest, accidental trauma, or brain hemorrhage and from one adolescent who died of meningoencephalitis. None of these five patients had evidence of liver disease and the results of their hepatic lipid analyses were used as control values.

The tissue was homogenized and extracted five times with diethyl ether:isopropyl alcohol (1:1, vol:vol). Portions of the combined lipid extracts were used to determine hepatic lipids by the following methods used routinely in our laboratory: total cholesterol and triglycerides as described by Ellefson and Caraway[7]; free fatty acids by the method of Dole[8]; and total phospholipids by the formation of molybdenum blue after oxidative digestion with 70% $HClO_4$:10 N H_2SO_4 (1:9, vol:vol), using a modification[7] of the Gomori[9] method. Neutral lipids were separated by thin-layer chromatography on silica gel H using hexane:diethyl ether:acetic acid (60:12:1, vol:vol:vol) as developing solvent system. Free fatty acids were eluted from silica gel with diethyl ether:methanol (9:1, vol:vol) and methylated directly with diazomethane[10] for analysis by gas chromatography. Triglycerides were separated and eluted from silica gel similarly and were saponified, and the resultant fatty acids were methylated with diazomethane for analysis by gas chromatography. Chromatographic separation was accomplished using a 2-meter column packed with 15% diethyleneglycol succinate on 80/100 Chromosorb W (AW) at 185°C with helium as carrier gas. Fatty acid methyl esters were identified in chromatograms by comparing retention times with known standards and were quantitated by planimetry.

Individual phospholipid fractions were separated by thin-layer chromatography on silica gel H, using chloroform:methanol:water (65:25:4, vol:vol:vol) as

Table 2.—**Hepatic Lipids in Reye-Johnson Syndrome and in Acute Encephalopathy Without Fatty Liver***

Patient	Diagnosis	Cholesterol	Triglycerides	Free fatty acids	Phospholipids
2	Reye-Johnson syndrome	13.9	119.1	17.6	28.7
3	Reye-Johnson syndrome	15.3	125.2	12.5	26.8
4	Reye-Johnson syndrome	22.1	128.3	15.6	28.2
5	Reye-Johnson syndrome	27.3	261.2	14.9	27.0
6	Reye-Johnson syndrome	14.4	230.8	6.2	22.0
	Mean	18.6	172.9†	13.4	26.5†
	SD	5.9	67.7	4.4	2.6
8	Acute encephalopathy	9.9	17.4	52.0	26.8
9	Acute encephalopathy	13.8	14.2	21.8	30.1
10	Cardiac arrest	17.2	15.3	18.0	35.2
11	Car accident	10.4	27.8	8.5	29.9
12	Meningoencephalitis	8.7	4.2	8.9	49.0
13	Cardiac arrest	14.4	50.6	69.5	40.6
14	Brain hemorrhage	16.5	34.5	33.3	34.0
	Mean	13.4	26.5†	27.5	37.7†
	SD	3.7	17.8	25.4	7.4

*Values are given as μmol/g of fresh liver.
†$P<0.01$, comparing Reye-Johnson syndrome group (patients 2 to 6) with control group (patients 10 to 14).

developing solvent system, were eluted from silica gel with methanol:hydrochloric acid (99:1, vol:vol), and were quantitated by the same method used for determining total phospholipids. Phospholipid fractions were identified on thin-layer chromatograms by comparing chromatographic mobilities with commercially available standards and by using spray reagents and colorimetric reactions, as previously described.[11]

RESULTS

The results of total cholesterol, triglyceride, free fatty acid, and phospholipid determinations in liver tissue from patients with Reye-Johnson syndrome and acute encephalopathy, and from controls are shown in Figure 1.

Total Cholesterol.—The mean concentration for total hepatic cholesterol was slightly higher in Reye-Johnson syndrome and lower in acute encephalopathy when compared with the mean control value (Table 2). These differences, however, were not statistically significant.

Triglycerides.—The mean liver triglyceride concentration was markedly elevated in Reye-Johnson syndrome and averaged nearly seven times the mean triglyceride content found in control liver tissue (Table 2). On the other hand, the mean triglyceride value in acute encephalopathy livers was lower than that found in the control livers.

The percentage distribution of specific fatty acids in the triglyceride fractions is shown in Table 3. The ratio of palmitate to oleate was 2.5 in acute encephalopathy, 1.2 in Reye-Johnson syndrome, and 0.7 in controls.

Table 3.—**Fatty Acid Composition of Hepatic Triglycerides in Reye-Johnson Syndrome, in Acute Encephalopathy, and in Controls***

Fatty acid (carbon chain length:unsaturated bonds)	Reye-Johnson syndrome		Acute encephalopathy		Control	
	Patient 4	Patient 6	Patient 8	Patient 9	Patient 11	Patient 13
Caprylic (8:0)	...	...	...	Trace	...	...
Capric (10:0)	5.8	1.3	...	5.7	...	...
Lauric (12:0)	4.4	3.3	2.2	2.5	0.8	Trace
Myristic (14:0)	9.0	6.6	4.4	5.0	4.7	2.1
Palmitic (16:0)	23.0	24.3	39.4	49.4	29.9	20.0
Palmitoleic (16:1)	12.8	13.9	7.3	4.7	8.0	10.4
Stearic (18:0)	1.2	2.4	3.6	3.5	5.5	2.6
Oleic (18:1)	15.2	27.3	23.4	14.8	32.1	44.6
Linoleic (18:2)	7.6	7.3	2.2	1.3	11.7	17.3
Linolenic (18:3)	Trace	...	Trace	...	...	1.0
Arachidic (20:0)	...	...	...	...	...	...
Arachidonic (20:4)	Trace	...	10.2	...	...	...
Unidentified	20.9	13.7	7.3	13.2	7.3	2.1

*Values for fatty acids are given as percent of total.

Table 4.—**Hepatic Free Fatty Acids in Reye-Johnson Syndrome, in Acute Encephalopathy, and in Controls***

Fatty acid (carbon chain length:unsaturated bonds)	Reye-Johnson syndrome		Acute encephalopathy		Control	
	Patient 4	Patient 6	Patient 8	Patient 9	Patient 11	Patient 13
Caprylic (8:0)	...	...	...	...	...	...
Capric (10:0)	5.0	...	Trace	Trace	...	...
Lauric (12:0)	2.5	...	0.4	Trace	1.1	Trace
Myristic (14:0)	5.0	Trace	1.3	1.5	2.8	2.3
Palmitic (16:0)	45.0	55.0	22.9	34.1	19.9	42.7
Palmitoleic (16:1)	2.5	5.0	5.7	3.7	7.1	4.7
Stearic (18:0)	12.5	15.0	7.9	7.4	6.6	2.3
Oleic (18:1)	20.0	20.0	39.2	41.5	29.0	43.7
Linoleic (18:2)	7.5	5.0	21.1	11.9	15.0	4.2
Linolenic (18:3)	...	...	Trace	...	0.7	...
Arachidic (20:0)	...	...	...	...	1.4	...
Arachidonic (20:4)	...	...	...	...	7.8	...
Unidentified	...	...	1.3	...	8.6	...

*Values for free fatty acids are given as percent of total.

Free Fatty Acids.—The mean free fatty acid concentration in liver tissue from patients with acute encephalopathy was nearly three times that found in Reye-Johnson syndrome liver tissue (Table 2). The ratio of palmitic acid to oleic acid in hepatic free fatty acids was 0.7 in acute encephalopathy, 2.5 in Reye-Johnson syndrome, and 0.8 in controls (Table 4).

Phospholipids.—The mean total hepatic phospholipid concentration was low in both Reye-Johnson syndrome and acute encephalopathy compared with the mean control value (Table 2); however, the difference was statistically significant only between Reye-Johnson syndrome and controls.

The results of quantitative analyses of individual phospholipids are shown in Table 5. The percentage distribution of the three major phospholipid fractions, lecithin (phosphatidyl choline and phosphatidyl serine), phosphatidyl ethanolamine, and sphingomyelin, was similar among livers from patients with Reye-Johnson syndrome and acute encephalopathy and from controls. However, when results were expressed in absolute molar values, lecithin content was lower in Reye-Johnson syndrome than in controls.

DISCUSSION

Fatty Liver in Reye-Johnson Syndrome.—Fatty liver is an essential but nonspecific pathologic feature of Reye-Johnson syndrome. It may occur also in a variety of clinical and experimental conditions including intoxications (for example, carbon tetrachloride, phosphorus, amatoxins, aflatoxins, hypoglycin A, orotic acid, and ethionine), nutritional deficiencies (for example, kwashiorkor and alcoholism), and certain disorders of amino acid metabolism. In contrast

Table 5.—**Hepatic Phospholipids in Reye-Johnson Syndrome, in Acute Encephalopathy, and in Controls***

Patient; diagnosis	Phospholipid†							
	Lecithin	PE	SPM	LPC	DPG	PG	PA	UPL
2; Reye-Johnson syndrome	50	27	8	1	0	1	1	0
3; Reye-Johnson syndrome	52	28	7	3	3	1	0	0
4; Reye-Johnson syndrome	52	22	9	1	2	2	0	13
5; Reye-Johnson syndrome	50	15	19	0	12	1	0	3
6; Reye-Johnson syndrome	47	24	5	1	1	1	1	0
8; Acute encephalopathy	46	23	7	2	1	2	1	4
9; Acute encephalopathy	56	18	7	5	4	2	4	3
10; Cardiac arrest	50	25	5	2	4	1	2	0
11; Car accident	43	25	6	1	2	3	1	3
12; Meningoencephalitis	37	16	6	5	5	4	3	5
13; Cardiac arrest	40	21	5	3	4	4	3	0
14; Brain hemorrhage	43	21	6	3	0	3	1	4

*All values are given as percent of total phospholipid phosphorus.
†Lecithin includes phosphatidyl choline and phosphatidyl serine; PE = phosphatidyl ethanolamine; SPM = sphingomyelin; LPC = lysophosphatidyl choline; DPG = diphosphatidyl glycerol; PG = phosphatidyl glycerol; PA = phosphatidic acid; UPL = unidentified phospholipids. Recovery of phospholipid fractions by thin-layer chromatography averaged 88% of total hepatic phospholipids.

to the situation in the conditions mentioned, in Reye-Johnson syndrome the etiology of fatty liver is unknown. In a few cases an association with aflatoxins,[12] pteridines,[13] and isopropyl alcohol[14] has been reported but in most patients with Reye-Johnson syndrome no exogenous toxin has been found. Recent reports of short-chain fatty acidemia[15] and of the existence of circulating endotoxins,[16] presumably derived from intestinal bacteria, suggest that endogenous toxins may play an etiologic role in some patients who have Reye-Johnson syndrome.

Histochemical studies[17] have shown increased levels of neutral lipids in the livers of patients with Reye-Johnson syndrome. The neutral lipid deposits appear usually as microdroplets that neither displace the nucleus nor distort the hepatocyte architecture. A similar morphology has been described in tetracycline hepatotoxicity and in the fatty metamorphosis of pregnancy. The appearance is different from that seen in other conditions associated with fatty liver, in which large lipid droplets often coalesce to form "lakes" that distort the hepatocyte. The significance of these histologic differences is not known.

Lipid analyses of fatty livers in patients with Reye-Johnson syndrome have shown a high triglyceride content. Norman and associates[4] reported hepatic triglycerides to be increased nearly threefold in Reye-Johnson syndrome. We found hepatic triglycerides to be increased nearly sevenfold in Reye-Johnson syndrome. Total hepatic cholesterol, free fatty acids, and phospholipids were not increased in our patients. These findings indicate that triglycerides account for most, if not all, of the lipid increment detected histochemically in the livers of patients with Reye-Johnson syndrome.

Total phospholipids were decreased significantly in livers of our patients with Reye-Johnson syndrome. This observation agrees with the data provided by Norman and associates[4] and by Pollack and associates.[6] Hepatic total phospholipids are decreased in carbon tetrachloride poisoning and in *d*-galactosamine-induced fatty liver in rats.[18] Lipoprotein formation and fatty acid oxidation seem to be influenced by the hepatic phospholipid concentration.[18] The fatty liver in Reye-Johnson syndrome may result from impaired triglyceride secretion into plasma or from decreased oxidation of fatty acids from hepatic triglycerides.

Quantitative analysis of individual phospholipid fractions indicated that hepatic lecithin was decreased to a greater extent than other phospholipids in Reye-Johnson syndrome. Lecithin is an important constituent of biomembranes.[19] Ultrastructural studies[20] have shown abnormalities of mitochondria in the livers of patients with Reye-Johnson syndrome. It is possible that these mitochondrial changes may be related to a decreased content of lecithin in mitochondrial membranes. Fatty acid oxidation is exclusively a mitochondrial process which, if impaired, might lead to the development of fatty liver in Reye-Johnson syndrome.

The fatty acid composition of hepatic triglycerides and free fatty acids was studied in two patients with Reye-Johnson syndrome, in two patients with acute encephalopathy, and in two controls. Palmitic acid was the most abundant free fatty acid found in the livers of patients with Reye-Johnson syndrome, whereas oleic acid predominated in acute encephalopathy and in control patients' livers. In addition, the percentage of hepatic distribution of other saturated fatty acids, was increased in one of the cases of Reye-Johnson syndrome when compared with that in acute encephalopathy and in controls.

The fatty acid composition of hepatic triglycerides, on the other hand, showed less striking differences in Reye-Johnson syndrome as compared with that in control patients. Oleate was the predominant triglyceride fatty acid in control patients' livers. The ratio of oleate to palmitate was nearly equal in Reye-Johnson syndrome, whereas palmitate was the predominant fatty acid in hepatic triglycerides from patients with acute encephalopathy.

These fatty acid profiles, although obtained from a limited number of samples, suggest that the relative hepatic distribution of saturated and monoenoic fatty acids may differ significantly in acute encephalopathy and in Reye-Johnson syndrome.

We were particularly interested in demonstrating increased amounts of short-chain fatty acids in liver samples obtained post mortem from patients who had Reye-Johnson syndrome. Preliminary studies using trimethylsilyl[21] derivatives failed to show any accumulation of short-chain fatty acids in our material. According to Trauner and her associates,[15] short-chain fatty acidemia is an early and transient phenomenon in Reye-Johnson syndrome. If similar changes are reflected in tissue such as liver, short-chain fatty acids may not be demonstrable in postmortem samples.[22]

Acute Encephalopathy Without Fatty Liver.—A mild to moderate increase in stainable neutral lipid in liver tissue from Glasgow and associates'[3] patients and in our own material from patients with acute encephalopathy without fatty liver suggested that such cases might represent a forme fruste of Reye-Johnson syndrome. But the results of hepatic lipid analyses reported here do not support such a hypothesis. There was no increase in hepatic triglycerides in patients with acute encephalopathy. Furthermore, free fatty acids in the liver were increased nearly threefold in

acute encephalopathy when compared with Reye-Johnson syndrome.

Sequential liver biopsies indicate rapid reversibility of the fatty changes in patients with Reye-Johnson syndrome who survive.[20] The patient reported by Glick[23] as an example of Reye-Johnson syndrome without fatty liver is difficult to accept because liver biopsy was done on the sixth hospital day when the patient was clinically recovered. It is possible that fatty liver also was present in Glasgow and associates'[3] cases and in our two patients with acute encephalopathy but that the condition had reverted to normal by the time the liver was examined. However, no evidence is available at present to indicate that fatty changes in liver are reversible in fatal cases of Reye-Johnson syndrome.

The discrepancy between the histologic findings of mild to moderate increase in stainable neutral lipid and the absence of increased triglyceride content in liver tissue from patients with acute encephalopathy remains unresolved. A considerable amount of neutral lipid exists in normal liver tissue that fails to stain with routine neutral lipid dyes. It has been suggested that this lipid is bound to proteins, forming lipoprotein complexes that are inaccessible to neutral lipid stains. Dissociation of lipoprotein complexes would release the lipid and render it accessible to neutral lipid stains without any actual increase in tissue lipid content.[24] The possibility also exists that increased stainable neutral lipid without concomitant increased triglyceride content may be a postmortem artifact.

What, then, is the relationship between acute encephalopathy without fatty liver and Reye-Johnson syndrome? Acute encephalopathy without fatty liver, or acute toxic encephalopathy, was recognized as a clinicopathologic entity before Reye and his colleagues[1] emphasized its association with visceral fatty changes. Lyon, Dodge, and Adams,[25] in their report of infants and children with acute encephalopathies of obscure origin, included detailed descriptions of the pathologic findings but did not mention fatty liver. Sporadic reports of acute encephalopathy with fatty liver, often associated with varicella, may be found in the literature as far back as 1929.[26] It is unlikely that Reye-Johnson syndrome would have been overlooked in the past, particularly if it had presented in epidemic form. A more plausible explanation is that Reye-Johnson syndrome is a new entity that has emerged recently to gain widespread recognition.

The biochemical data obtained from our patients lead us to conclude that, despite the clinical similarities and laboratory evidence of hepatic dysfunction in both syndromes, Reye-Johnson syndrome and acute encephalopathy represent different entities, possibly with different pathogenic and etiologic mechanisms.

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Wegener's Granulomatosis

Anatomic Correlates, A Proposed Classification

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Based on a 10-year experience with 50 patients who had Wegener's granulomatosis, a new classification is proposed based on anatomic site of involvement: upper airway or ear, nose, and throat (designated E), lung (L), and kidney (K). All combinations of ELK were seen. The system is offered as a unifying concept to embrace the terms midline granuloma, generalized or classic Wegener's granulomatosis, and limited Wegener's granulomatosis. Fourteen patients have died despite the use of corticosteroids and immunosuppressant therapy. Thirteen patients with renal involvement have survived for periods as long as 114 months; all are taking immunosuppressive agents.

Much controversy exists over the relationship of classic or generalized Wegener's granulomatosis, limited Wegener's granulomatosis, and midline granuloma. Until the etiology and pathogenesis of these conditions are elucidated, the question must remain unsettled. Nevertheless, patients who have necrotizing granuloma and vasculitis, with or without focal necrotizing glomerulitis, have similarities that make it attractive and provocative to view all of them as part of a single disease spectrum or continuum. In this broader concept we herein report a 10-year experience with 50 such patients, correlating the major sites of manifest anatomic involvement with salient laboratory findings and clinical course.

METHODS AND MATERIALS

Data on 50 patients, 28 men and 22 women, who had biopsy-proven necrotizing granuloma with vasculitis, with and without focal necrotizing glomerulitis, were analyzed. They were seen over a 10-year period ending in 1974. Patients ranged in age from 8 to 75 years. Six patients (12%) were 25 years of age or younger and 11 (22%) were older than 60. They were classified according to the major anatomic site of involvement: the upper airway or ear, nose, and throat were designated E, the lung L, and the kidneys K. Other less frequently involved sites included the orbit, peripheral and central nervous systems, heart, skin, and gastrointestinal tract. Erythrocyte sedimentation rate was performed by the Westergren method, rheumatoid factor by latex fixation, antinuclear antibody by immunofluorescence, and the lupus erythematosus clot test by the McGath modification. Serum immunoglobulins were determined by radioimmunodiffusion. Survivorship was calculated in months from the time of diagnosis. All biopsy specimens were subjected to special staining for microorganisms and were cultured for bacteria, mycobacteria, and fungi. Specifically excluded from analysis were patients with polymorphic reticulosis, also called midline malignant reticulosis or lymphomatoid granulomatosis, a disease often confused with Wegener's granulomatosis but having distinct pathologic features and clinical course.

*Deceased, July 5, 1975.

RESULTS

*Anatomic Sites**.—Each of the major sites—E, L, or K—may be involved alone or in combination except that the kidney cannot be involved alone because focal necrotizing glomerulitis is not specific for Wegener's granulomatosis. According to older terminology, the classic form of Wegener's granulomatosis could be designated by E, L, K in our system. Upper airway involvement alone would be designated by E and would approximate the old term, midline granuloma. Patients classified as EL or L would appear to coincide with the concept of limited Wegener's granulomatosis. Table 1 shows a categorization of patients according to the E, L, K scheme. Hereafter we shall refer to all patients as belonging to a class based on the E, L, K site designation.

By individual site, E was most frequently involved (37 patients), followed by L (35 patients) and K (23 patients). The skin was involved in eight patients, the orbit in six, peripherial nerves in seven, central nervous system in four, gastrointestional tract in three, and the heart in two. Renal involvement was documented by biopsy in 11 patients and at autopsy in 2 further instances. In 10 patients, renal involvement was diagnosed on the basis of classic findings of red blood cell casts and proteinuria. Orbital involvement was manifested by proptosis and was always associated with E. Because of such constancy, orbital involvement came to be looked upon as an extension of E. Skin involvement was documented by biopsy in seven patients and diagnosed clinically in one. Peripheral nerve disease usually took the form of mononeuritis multiplex. Central nervous system disease was manifested by delta wave focus on electroencephalography, cerebral dysfunction clinically, or cerebral hemorrhage. Although the disease in most patients did not progress beyond sites originally observed at first diagnosis, six patients developed other involvement at some time remote from the original diagnosis. One patient evolved from E to EL to ELK (patient 7), eventually dying after a 15-month course. Another patient (patient 39) evolved from E to EK over 18 months while on corticosteroids. Patient 26, with biopsy-proven E, evolved to EL proven by lung biospy 4 years later. Another patient (patient 28) evolved from E to ELK in 2 years. One patient (patient 50) had chronic nasal obstruction and discharge with polyarthritis 2 years before identification as EL. Another patient (patient 38) progressed from E to ELK with central nervous system and gastrointestinal involvement. These observations of evolution support the concept that the disease exists as a continuum.

Table 1.—**Wegener's Granulomatosis as Found at Various Anatomic Sites**

ELK class	Men	Women	Total
E	4	7	11
L	4	4	8
EL	7	1	8
EK	2	2	4
LK	3	2	5
ELK	8	6	14
Total	28	22	50

Fourteen patients (5 women, 9 men) are known to be dead, 10 from renal failure. Of the four without renal involvement, one (patient 3) died with gram-negative bacterial sepsis and one (patient 19) with massive pulmonary hemorrhage and *Pseudomonas* sepsis. Hodgkin's disease was the alleged cause of death of a 51-year-old man (patient 18) with L involvement. However, we have been unable to obtain tissue to confirm this. The original diagnosis, made on lung biopsy specimens, was corroborated by Dr. Averill Liebow. The last patient (patient 8) is known to be dead after surviving 92 months, but details of the cause of death could not be obtained.

Laboratory Findings.—Table 2 displays the salient laboratory findings. Defining anemia as a hemoglobin concentration of less than 11 g/dl for women and less than 12 g/dl for men, 28 patients were anemic. The anemia was usually mild, but levels in the range of 8 to 9 g/dl were recorded, particularly in more seriously ill patients with more extensive involvement. The peripheral blood smear showed schistocytes and burr cells in four patients and a regenerative or hemolytic pattern in seven. A leukoerythroblastic picture was seen in two patients. It appears that the erythrocytes are prominently affected in Wegener's granulomatosis, part of which may be due to physical trauma from the widespread damage of vascular endothelium consequent to vasculitis. Such abnormalities of the peripheral blood smear closely follow the presence of anemia.

A leukocyte count greater than $10,900/mm^3$ was found in 24 patients and was more frequently seen in patients with higher ELK classes. An eosinophil count greater than 5% was found in six patients, the highest being 11.5%. The highest total eosinophil count was $1,150/mm^3$. This finding emphasizes the infrequency of significant peripheral eosinophilia in Wegener's granulomatosis as compared with that found in Churg-Strauss syndrome.

*A detailed table identifying anatomic sites of involvement and describing specific clinical data is available on request from the first author.

Table 2.—**Laboratory Findings in Wegener's Granulomatosis**

ELK class	No. of patients	Sedimentation rate (>80 mm in 1 hour) No. tested	No. positive	Rheumatoid factor No. tested	No. positive	Anemia No. tested	No. positive	Leukocytosis No. tested	No. positive
E	11	10	3	8	1	11	0	11	1
L	8	8	2	5	1	8	4	8	3
EL	8	8	3	1	1	8	3	8	3
EK	4	4	3	3	1	4	4	4	3
LK	5	5	4	5	3	5	4	5	4
ELK	14	14	13	13	8	14	13	14	10
Total	50	49	28	35	15	50	28	50	24

Antinuclear antibody determinations were positive in only 3 of 29 patients and all were of a mixed pattern. The lupus erythematosus clot test revealed rosettes of neutrophils in 2 of 37 patients but none were frankly positive. These two suggestive tests were associated with negative results for antinuclear antibody.

The rheumatoid factor was positive in 15 of 35 patients. Titers ranged from 0 to 1:2,560. The presence of rheumatoid factor correlated strongly with renal involvement (12 of 21 positives).

Erythrocyte sedimentation rates of 80 mm in 1 hour or greater were recorded in 28 patients. Thirteen of 14 ELK patients had such elevations. There appeared to be an increasing likelihood of high sedimentation rates in higher ELK classes.

Fauci and associates[1] have reported elevations of IgA as a feature of Wegener's granulomatosis. We have immunoglobulin data on 21 patients (Table 3). Immunoglobulin G was elevated in four, IgM in six, and IgA in four. Two patients had elevated immunoglobulin E. We[2] have reported this finding previously, including three additional patients with similar elevations not included in this analysis.

Table 3.—**Immunoglobulin Levels in Patients With Wegener's Granulomatosis**

Patient no.	ELK class	Immunoglobulin IgG	IgM	IgA	IgE
5	LK	7.2	0.43	1.03	
12	E	15.0*	0.49	3.5*	
15	E	14.0	4.10*	2.05	
16	EL	14.2	0.86	2.1	
18	L	7.6	0.9	1.18	
19	L	16.0*	1.15	2.25	
20	LK	9.8	1.75*	1.5	
21	L	7.8	1.5*	1.8	
23	E	16.0*	0.5	3.65*	1,925*
24	E	13.9	0.81	0.6	
29	E	9.5	0.64	2.5	
31	ELK	9.0	0.84	1.15	
33	EK	9.4	0.53	1.25	
34	E	9.1	0.89	1.5	
38	ELK	11.3	0.42	5.0*	
40	ELK	6.15	1.2	1.37	
44	LK	15.58*	0.37	4.25*	783*
46	ELK	12.13	2.01*	2.2	
47	E	8.3	1.19	1.43	409
48	ELK	13.67	1.44*	2.24	
49	ELK	7.2	0.64	1.5	1,364*

*Above normal range. Normal values: IgG, 6.4 to 14.3 mg/ml; IgM, 0.2 to 1.4 mg/ml; IgA, 0.3 to 3.0 mg/ml; IgE, 6 to 780 ng/ml.

TREATMENT AND SURVIVAL

Therapy varied greatly in the 10-year period during which this series was studied. The corticosteroid used in most cases was prednisone, with usual starting doses in the range of 60 to 80 mg/day, occasionally given on alternate days. Immunosuppressive agents used included cyclophosphamide (29 patients), azathioprine (11 patients), chlorambucil (5 patients), and busulfan (2 patients). It is not within our scope to report this aspect in great detail. Experience accumulated over this period has allowed us to make a few observations and generalizations. First, both corticosteroids and immunosuppressive agents are effective in treating Wegener's granulomatosis. However, they are not uniformly effective, as attested to by the fact that all deaths have occurred while the patients were taking such agents. The fact that 13 patients survived with renal involvement for up to 114 months after diagnosis, all on one or another immunosuppressive agent, speaks highly for the efficacy of such drugs.

Corticosteroids alone seem to be insufficient to control renal involvement, whereas such agents more frequently control lesions involving E or L. One patient seen with EK after closure of this series has experienced reversal of his disease in both sites while taking 60 mg of prednisone on alternate days over 18 months.

Two patients died of sepsis and another almost died of this complication. As one would expect, cortico-

steroids and immunosuppressive agents, used together, make the patient vulnerable to infectious complications—particularly the very ill patient with large portals of entry due to affected skin and respiratory epithelium. Because the action of immunosuppressive agents is delayed for periods of 2 to 3 weeks, we believe that corticosteroids should be used alone in the early treatment of severely ill patients, immunosuppressive agents being introduced after the patient has been stabilized, whereupon the corticosteroids are slowly withdrawn. In patients who have more indolent courses, we would elect to start administering immunosuppressive drugs alone.

Consideration must be given to the age of the patient because of long-term potential consequences of immunosuppressive therapy. All women ceased menstruating permanently while on cyclophosphamide; this drug was used generally in a dose of 2 mg/kg per day and at this dose was relatively safe, the chief side effects being sterility, menopause, and hair loss. No serious instance of hemorrhagic cystitis was encountered with oral use of this agent.

The efficacy of immunosuppressive agents such as cyclophosphamide has not, to our knowledge, been subjected to a controlled prospective study. However, reports of long-term survival of patients with renal involvement treated with such agents provide such strong evidence as to make withholding of these drugs from likely candidates unwise. The optimal dose of these drugs might well be subjected to prospective analysis, but the relative infrequency of the disease would require many years to develop significant data. This fact suggests the need for cooperative studies among large medical centers.

Finally, two patients with isolated lesions of the lung are stable without other therapy after surgical resection.

DISCUSSION

Although Klinger[3] alluded to the disease in 1931, it was Wegener[4] in 1939 who first clearly defined the entity that bears his name. The criteria he set down were necrotizing granuloma with vasculitis of the upper and lower respiratory tracts, systemic vasculitis, and focal necrotizing glomerulitis. In 1954, Godman and Churg[5] detailed the pathology based on a study of seven patients. Early experience suggested that the disease ran a uniformly rapid course ending in death within a few months. In 1966, Carrington and Liebow[6] proposed the concept of "limited Wegener's granulomatosis," which embraced those patients in whom the lung was chiefly affected in the absence of renal involvement. These patients experienced a relatively benign course compared with the classic or generalized form. Eight of their 16 patients were living from 4 to 150 months after the onset of symptoms. Additional strength was given to this concept in a report of four similar patients by Cassan, Coles, and Harrison[7] in 1970. An older term, lethal midline granuloma, bears on this discussion. This term, which antedates Wegener's original report, probably included not only patients with typical necrotizing granuloma and vasculitis consistent with Wegener's granulomatosis but also others with polymorphic reticulosis, specific infectious granulomas, lymphomas, and other neoplasms. In modern parlance this term has insufficient specificity to be meaningful.

The conceptional dilemma underlying all of these terms (Wegener's granulomatosis, limited Wegener's granulomatosis, and midline granuloma) is whether they are related or separate entities. Differences in clinical course would lend weight to the argument that they are separate. There appear, however, to be more compelling considerations that favor a unified concept which incorporates all three diseases into a continuum or spectrum. Necrotizing granuloma with vasculitis is identical in E or L and thus forms the common pathologic basis for diagnosis. Focal necrotizing glomerulitis is the hallmark of renal involvement when associated with the pathologic changes mentioned above. The data we present show that all possible combinations of anatomic sites can be involved. Evolutions of patients from E through L to K support a unified concept, as does the response of the lesions to similar therapeutic agents such as corticosteroids and immunosuppressants. Commonly shared laboratory findings also support a common pathogenesis. Those patients remaining stationary at E or L, not evolving to K, may reflect a different tempo of disease rather than different diseases. In addition, treatment administered may have prevented progression.

In the framework of a unified concept designated by the ELK scheme, a number of interesting hypotheses and questions are raised. The frequency of involvement of E and L and the observed evolution of E through L to K suggest that the respiratory tract may be the portal of entry of some as yet unknown provocative agent or agents. It would also seem reasonable that the respiratory epithelium serves as the first barrier to such an agent. The fact that immunoglobulin E can be found in increased amounts has a number of ramifications, among which might be the possibility that some predisposing allergic host factor exists. Increase in immunoglobulin A, reported by others,[1] has been cited as evidence suggesting that chronic

antigenic stimulation of surface membranes may be a possible cause. Our data on immunoglobulins are at some variance with these reports but the hypothesis is nevertheless attractive. Why does the disease remain localized in some patients and disseminate widely with K involvement in others?

It may well be that a number of different agents or pathogenic mechanisms will ultimately be uncovered within this disease known as Wegener's granulomatosis. In the light of present knowledge, we believe that it is convenient to study such patients within the unified concept embraced by the ELK classification system. In addition, ELK presents an obvious and easily remembered mnemonic.

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A Significant Interaction Between Metronidazole and Warfarin

Interaction between metronidazole (Flagyl) and warfarin had been suggested based on the disulfiram-like effect of metronidazole and the known interaction of warfarin and disulfiram. This case report confirms that this interaction is clinically significant in man.

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Interactions between coumarin and other drugs are among the clinically most important drug reactions in medicine. In recent years, several new interactions with warfarin in particular have been described and the mechanisms responsible have been clarified.[1-5] O'Reilly,[6] in an experimental study, has reported a significant increase in prothrombin time response and warfarin blood levels in normal volunteers taking metronidazole (Flagyl) and warfarin compared with warfarin alone. This report confirms that this interaction is clinically significant in man.

REPORT OF CASE

This 31-year-old woman was hospitalized with a history of sudden pain behind the left knee, occurring as she arose from a chair the day before admission. This pain became so severe that walking was difficult and she could not straighten her leg fully. Excision of her mitral valve and insertion of a 3M Starr-Edwards mitral ball valve prosthesis had been done 6 years previously for mitral valve stenosis and incompetence. She had been maintained on oral anticoagulant therapy with warfarin since surgery. Anticoagulant therapy had been complicated during that period by cerebral emboli 2 years and 3 years before admission, both episodes resolving without residual effects. She underwent a vaginal hysterectomy 4 months before this admission because of menstrual irregularity while taking anticoagulants. There had been no serious bleeding episodes and her prothrombin time was well controlled at home with a dosage of 5 mg of warfarin each day for 3 days followed by 7.5 mg the fourth day, and then repeating the cycle. Her prothrombin time was last checked at home about 2 weeks before admission.

For 1 week before admission she had noted excessive bruising of her legs. She had been started on metronidazole (Flaygl), 750 mg/day, for trichomonal vaginitis 17 days before admission; she was given instructions and sufficient medicine for a 10-day course of therapy. Her only other medications included propoxyphene occasionally, an oral iron-containing preparation, and warfarin.

Several ecchymoses were noted on both legs and there was obvious hemorrhage into the subcutaneous tissue of the left lower extremity. The left calf was swollen but neither the venous pattern nor local skin temperature was increased. She had had no hematuria or melena.

A prothrombin time done in the emergency room the day before admission was 147 seconds (normal, 17 to 19 seconds). She had been given 15 mg of vitamin K_1 at that time. After admission the next day, her prothrombin time was 46 seconds. No further vitamin K was given and on the third day after admission her prothrombin time was 23 seconds. She was restarted on oral warfarin because she had one brief cerebral embolus, which also cleared without residual effects in the first week after reinstitution of warfarin therapy. Since that time oral anticoagulation has been maintained with warfarin, prothrombin times have continued to be in a satisfactory range, and there have been no further complications.

DISCUSSION

In general, drug interactions involve modification of drug metabolism (drug pharmacokinetics) or modification of drug action at receptor sites (drug pharmacodynamics).[7] Coumarin interactions with other drugs involve several of these specific mechanisms—for example, absorption (cholestyramine resin),[8] binding (phenylbutazone, chloral hydrate),[9-11] biotransformation (barbiturates via enzyme induction and resultant acceleration of coumarin metabolism),[12] and enzyme inhibition with resultant potentiation of coumarin effect (disulfiram).[13] There are direct effects on synthesis or catabolism of the vitamin K-dependent coagulation factors (oral contraceptives, vitamin K)[14,15] and, finally, platelet inhibitor agents in patients taking oral coumarins may impair normal hemostatic mechanisms sufficiently to cause bleeding.

Mechanisms of drug interaction have been clarified to a point where a lack of therapeutic effect or a toxic effect is more readily understood. About 97% of the warfarin present in the body is bound to protein and, hence, displacement of even small amounts of warfarin by an interacting drug greatly increases the concentration of free drug available to the receptor site.[16] Serious bleeding with this type of coumarin interaction is well documented.[10] The effect of agents that displace coumarin from binding sites may be transient, as has been demonstrated in recent years for chloral hydrate, where an increase in free warfarin accelerates warfarin metabolism and may result in bleeding only early in the sequence of use of the drugs. As pointed out by Koch-Weser,[17] the sequence of onset and subsidence of potentiation with binding interaction is complex and depends on the metabolic half-life of both the coumarin and the displacing drug. The clinical significance of enzyme induction (induction of the hepatic microsomal enzyme systems) with coumarins relates both to inability to obtain a therapeutic effect when coumarin metabolism is accelerated without increasing the maintenance dose and to the danger of bleeding once the inducing agent is stopped. Both possibilities apply to hospitalized patients in particular, for whom use of sedatives and hypnotics is common. Bleeding may also result when a drug that decreases the rate of metabolism of warfarin is given with warfarin.[13] Blood concentration of warfarin is increased and the prothrombin time prolonged without a change in the maintenance dose of warfarin. For any one person, the results of enzyme induction seem fairly reproducible but there are, normally, wide differences among individuals in microsomal enzyme activity, and susceptibility to induction is determined genetically.[7,18]

In 1973, O'Reilly[13] demonstrated the interaction of warfarin and disulfiram in man and proposed that the microsomal enzymes responsible for the metabolism of warfarin are inhibited by disulfiram, an effect leading to higher blood levels of warfarin and an enhanced anticoagulant effect. Disulfiram inhibits aldehyde dehydrogenase[19] and also has been shown to inhibit hepatic dopamine β-hydroxylase[20] as well as to interfere with the metabolism of diphenylhydantoin and antipyrine.[21] Both these last drugs share with warfarin the capacity for biotransformation by hydroxylation.

A disulfiram effect of metronidazole has been suggested.[7] On this basis, in 1975, O'Reilly[6] postulated an interaction between warfarin and metronidazole. He reported significant increase in prothrombin time response and warfarin blood levels in eight normal subjects receiving both drugs compared with the response in the same subjects on warfarin alone. The dose of metronidazole used was 750 mg orally, the same as in our patient. Based on this information it would seem probable that the mechanism of interaction is indeed inhibition of the metabolism of warfarin by metronidazole.

Our patient had marked bruising within 10 days of starting metronidazole, as well as significant elevation of her prothrombin time and a serious enough bleeding episode to require hospitalization. All of this occurred against a background of 6 years on warfarin without a bleeding problem. Speculative interpretation of what are often complicated events does not, in a single case, establish an unequivocal drug interaction but, in this instance, other possible drugs and intercurrent events were clearly identified and it did not seem feasible that they were implicated in any way. Considering the background of experimental work with these drugs, the clinical implications are clear. It is, of course, possible that not all persons taking warfarin and metronidazole concurrently will demonstrate the interaction, just as seems the case in the interaction between disulfiram and warfarin.[13] There are probably individual differences in the ability to inhibit hepatic microsomal enzymes.[21]

If, as claimed, a single 2-g dose of metronidazole can eradicate trichomonal infection, this therapeutic approach might prove an alternative to the conventional recommended dosage in patients taking warfarin.[22]

In addition, sodium warfarin is a racemic mixture of two optical enantiomorphs—S(−)-warfarin(so-called S warfarin) and R(+)-warfarin (R warfarin). S warfarin is the more potent anticoagulant in man, although in man it is eliminated more rapidly than R

warfarin.[23] It has also been shown that the metabolic products of R and S warfarin differ.[24] R warfarin is oxidized to 6-hydroxywarfarin and eventually reduced to warfarin alcohols, whereas S warfarin forms predominantly 7-hydroxywarfarin. S warfarin may derive its greater potency from intrinsic activity of the drug, and that increased activity may well result from its stereospecificity.[23] The interaction of metronidazole and warfarin seems stereospecific—that is, the reaction occurs with the more potent S warfarin enantiomorph.[6] Hence, it has been suggested that the reaction might be avoided by using the R warfarin instead of the racemic warfarin mixture.

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Ultrastructure of Ischemic Contracture of the Left Ventricle ("Stone Heart")

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Myocardial biopsies from two patients who had developed "stone heart" (myocardial rigor mortis; ischemic contracture of the left ventricle) were studied by electron microscopy. The ultrastructure of tissue in stone heart, though ischemic in nature, differed from that of classic myocardial infarction in some respects. Apart from depletion of glycogen and distension of the sarcoplasmic reticulum and T-tubules, myofibrillar degeneration was much more widespread. Mitochondrial degeneration with active lysosomal autodigestion, disruption of the microcirculation, and lymphedema were prominent changes also observed. In the light of known clinical and experimental observations, our findings suggest that stone heart is an accelerated form of ischemic injury occurring in vulnerable (hypertrophied) hearts and is probably related to ischemia-triggered release of endogenous catecholamines.

Ischemic contracture of the left ventricle (myocardial rigor mortis[1] or stone heart[2]) can be produced experimentally and has been observed clinically.[1-7] Myocardial rigor signifies irreversible ischemic damage of the heart. It is characterized by tetanic contraction of the left ventricle with almost total obliteration of the ventricular chamber and blanching of the subendocardial muscle.[2,6] The spastic heart cannot be induced to relax by any form of pharmacologic intervention or by prolonged cardiopulmonary bypass support and manual massage.[3]

Although stone heart was reported by Cooley and associates[2] as a rare complication of cardiac surgery, occurring in only 13 (0.3%) of 4,732 patients who had undergone a variety of open-heart procedures, it accounted for over 25% of the operative deaths from acute myocardial failure.[2] These 13 previously reported cases of stone heart had many common clinical and pathologic features. All patients were in either functional Class III or Class IV heart failure (New York Heart Association classification), and 11 of the 13 patients underwent open-heart surgery for correction of severe aortic valvular disease. Evidence of advanced left ventricular hypertrophy was present in every case, the average heart weight being 782 g.[3] The main histologic findings consisted of diffuse interstitial fibrosis and hypertrophied muscle fibers.[2,3]

The availability of surgical biopsy material from two recent cases of stone heart afforded us an opportunity to study the ultrastructure of tissue in myocardial rigor and formed the basis of this report.

MATERIALS AND METHODS

Myocardial biopsies were obtained from two men, 62 and 56 years old, who had developed the condition of stone heart at open-heart surgery with cardiopulmonary bypass for replacement of stenotic aortic valves. Both patients had anatomically moderately severe but clinically asymptomatic coronary atherosclerosis. Normothermic ischemic arrest of the heart was accomplished by cross-clamping of the aorta. The total ischemic times were 80 and 100 minutes, respectively, when stone heart

This investigation was supported in part by Research Grant HL-17269 from the National Heart and Lung Institute, National Institutes of Health, Public Health Service.
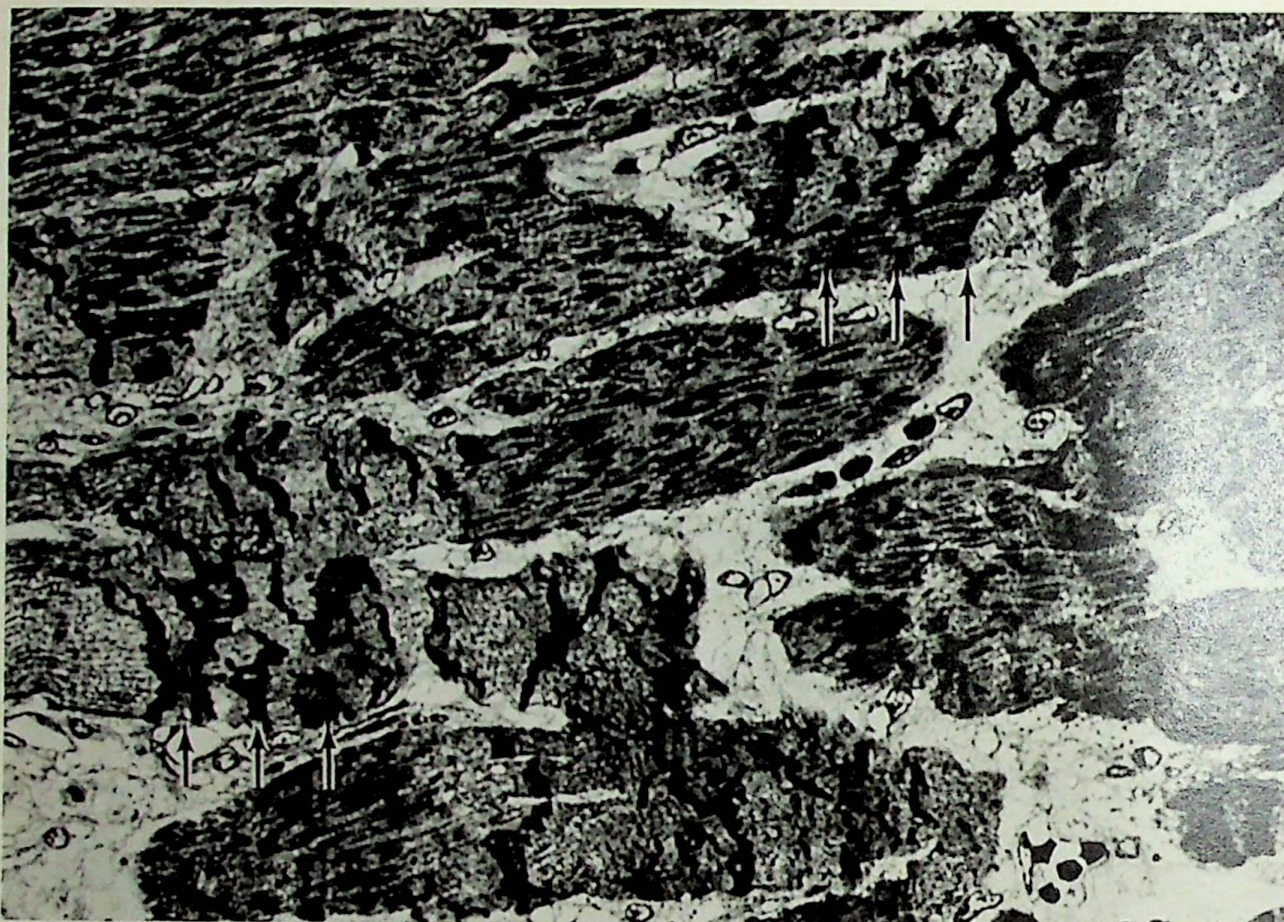
Fig. 1. Photomicrograph of myofibrillar degeneration. Note interstitial fibrosis and hypertrophied muscle fibers with contraction bands (*arrows*). (Azure II and methylene blue; ×1,000.)

Fig. 2. Photomicrograph of myocytolysis. Note dissolution of myofilaments and intracellular accumulation of pigmented lysosomal bodies (*arrows*). (Azure II and methylene blue; ×1,000.)
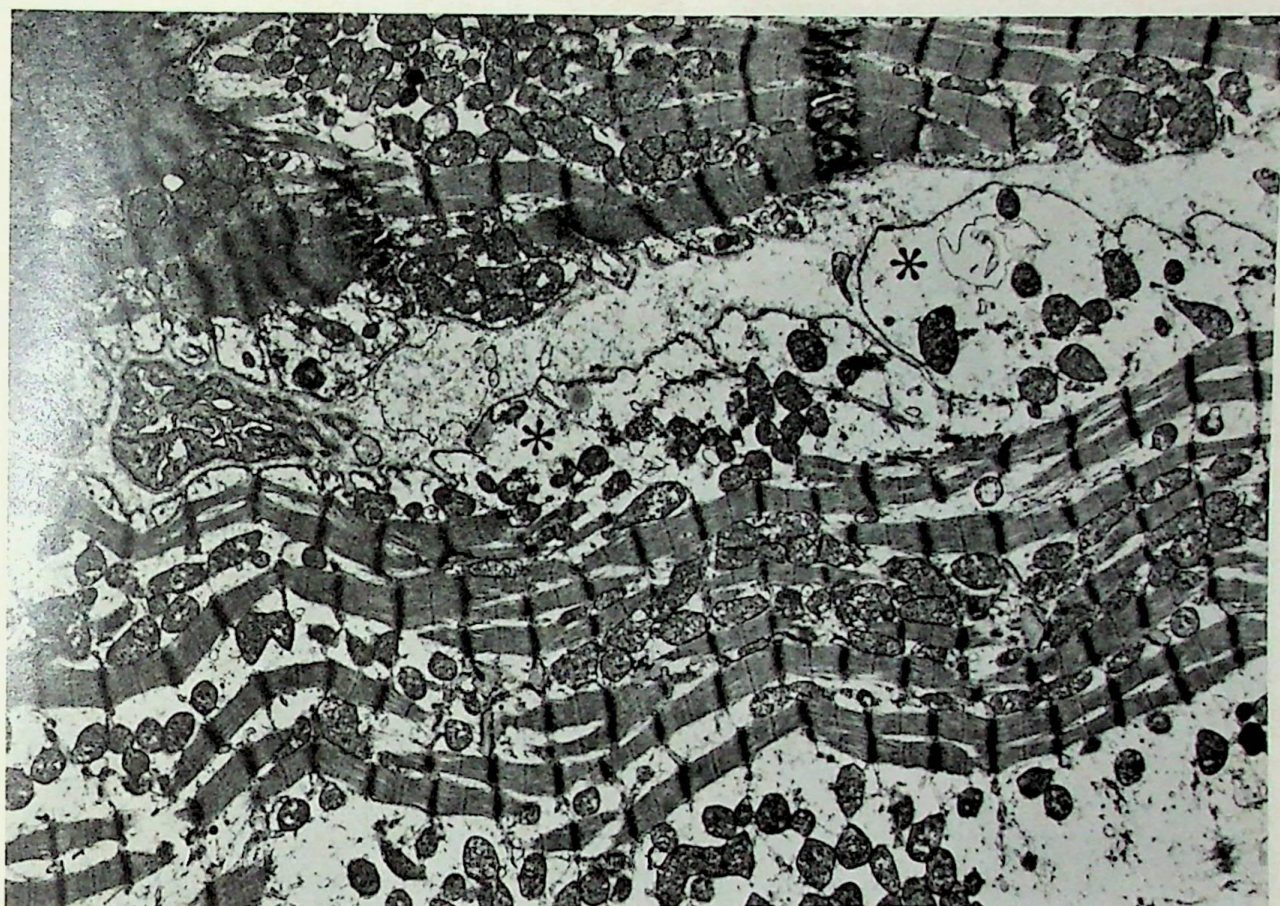
Fig. 3. Electron micrograph of stone heart. Note supercontracted fiber in left upper corner of figure and intracellular edema with subsarcolemmal blebs (*) of the fiber in lower half of figure. (Uranyl acetate and lead citrate; ×6,200.)
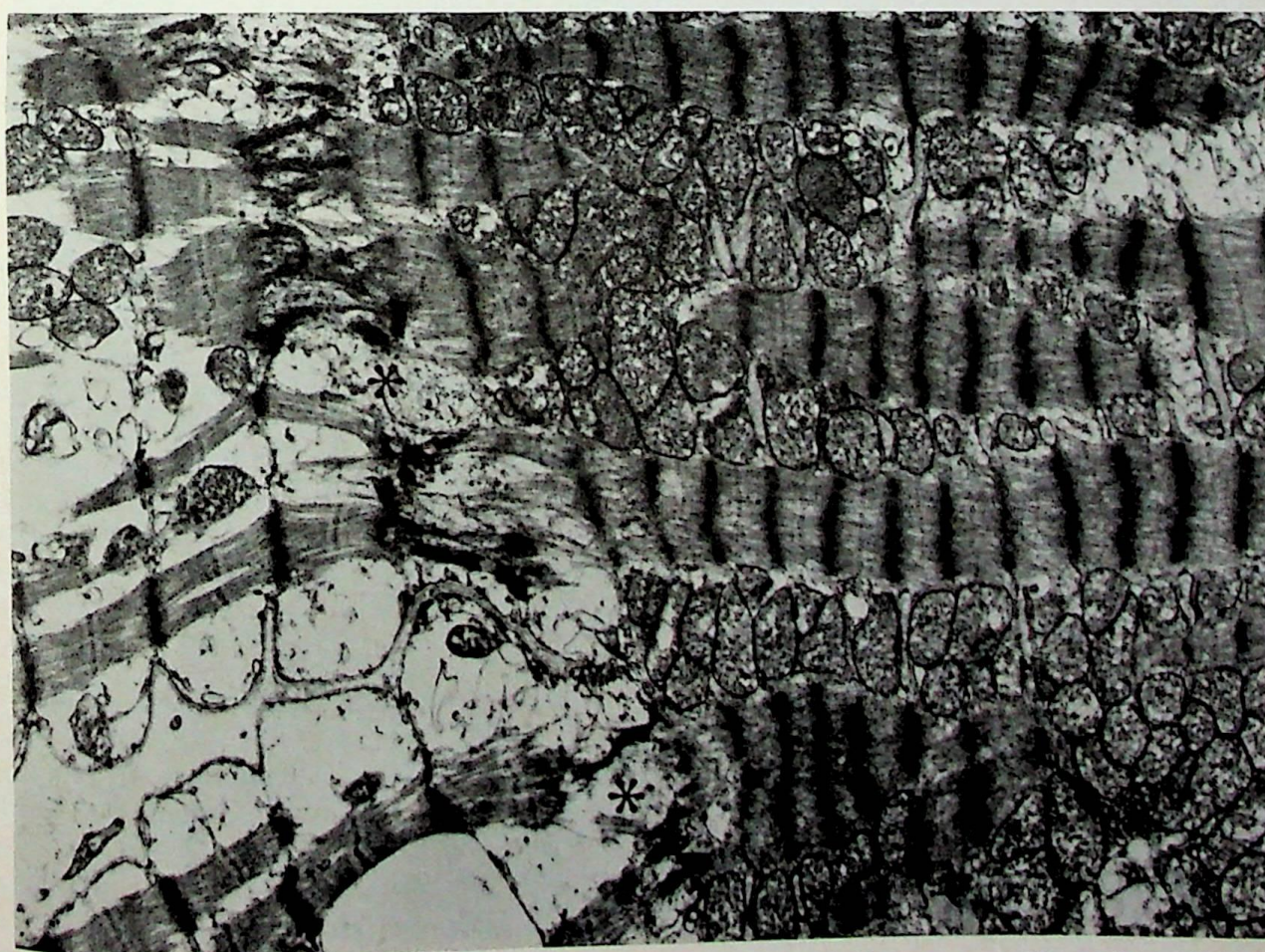
Fig. 4. Electron micrograph of stone heart. Note supercontracted fiber in right of figure, separated from adjacent fiber by torn intercalated disk (*). (Uranyl acetate and lead citrate; ×9,300.)
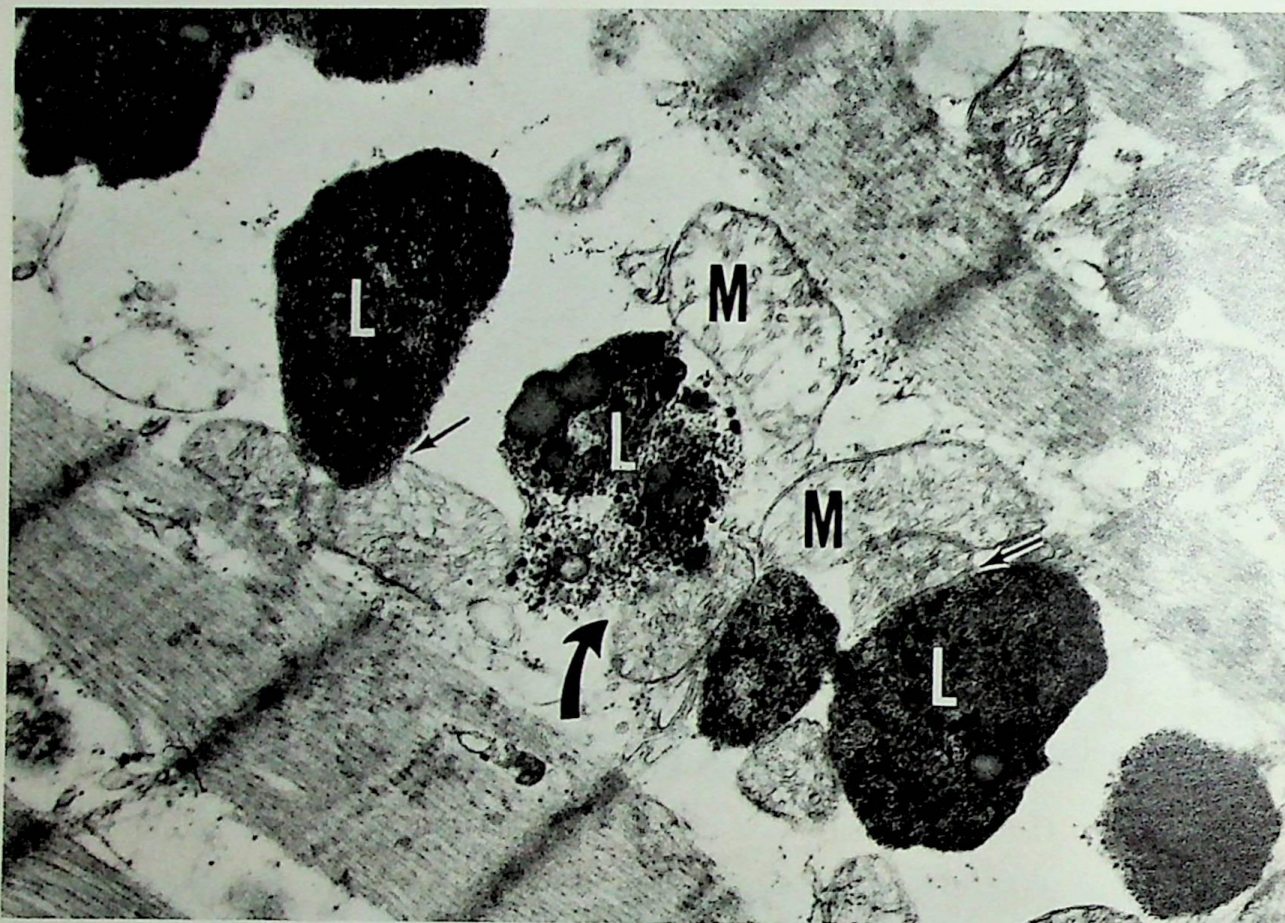
Fig. 5. Electron micrograph of stone heart. Note group of large secondary lysosomal bodies (*L*) filled with flocculent electron-dense material and in close contact (*small arrows*) with degenerated mitochondria (*M*). *Curved arrow* points to mitochondrion with disrupted limiting membrane. These lysosomal bodies are found outside nuclear pole areas. (Uranyl acetate and lead citrate; ×32,000.)

developed in these two patients. All attempts to reverse the myocardial rigor failed. At autopsy, the hearts (575 and 750 g, respectively) remained firmly contracted and there was almost total obliteration of the ventricular cavities.

In each case, full-thickness myocardium was taken from the anterior wall of the left ventricle as soon as the surgeons recognized that myocardial rigor had occurred. The tissue was cut into 1-mm cubes and fixed by immersion in phosphate-buffered 3% glutaraldehyde for 3 hours. After several rinses in the phosphate buffer, the tissue was postfixed in buffered 1% osmium tetroxide for 1 hour, dehydrated in graded alcohol solutions, and embedded in araldite. Sections were cut on a Porter-Blum MT-2 ultramicrotome with a diamond knife. Semithin (1-μ) sections were stained with methylene blue-azure II and thin sections were stained with uranyl acetate and lead citrate and examined in an RCA EMU-4 electron microscope. To demonstrate lysosomal activity, the glutaraldehyde-fixed specimens were cut at 40 μ and incubated in the reaction media for acid phosphatase by the Gomori technique[8] for both light and electron microscopy.

RESULTS

Identical morphologic abnormalities were seen in both cases of stone heart, although the severity of changes varied somewhat among the individual specimens examined. Apart from depletion of glycogen granules and widening of sarcoplasmic reticulum and T-tubules, the main pathologic changes involved the contractile elements, the mitochondria, and the microcirculation.

Myofibrillar Degeneration.—The individual myocardial fibers were markedly hypertrophic and appeared more widely separated because of interstitial fibrosis. The orderly cross-striation of the fibers was interrupted by dense "contraction bands" (Fig. 1). In some areas, there was widespread dissolution of myofibrils and intracellular accumulation of small osmophilic lysosomal bodies (Fig. 2). Frequently, adjacent fibers showed markedly contrasting states of

Fig. 6. Photomicrograph of large autophagic vacuole with double-layered wall (*arrows*), containing degenerated mitochondria and naked membrane structures (*). (Uranyl acetate and lead citrate; ×18,600.)
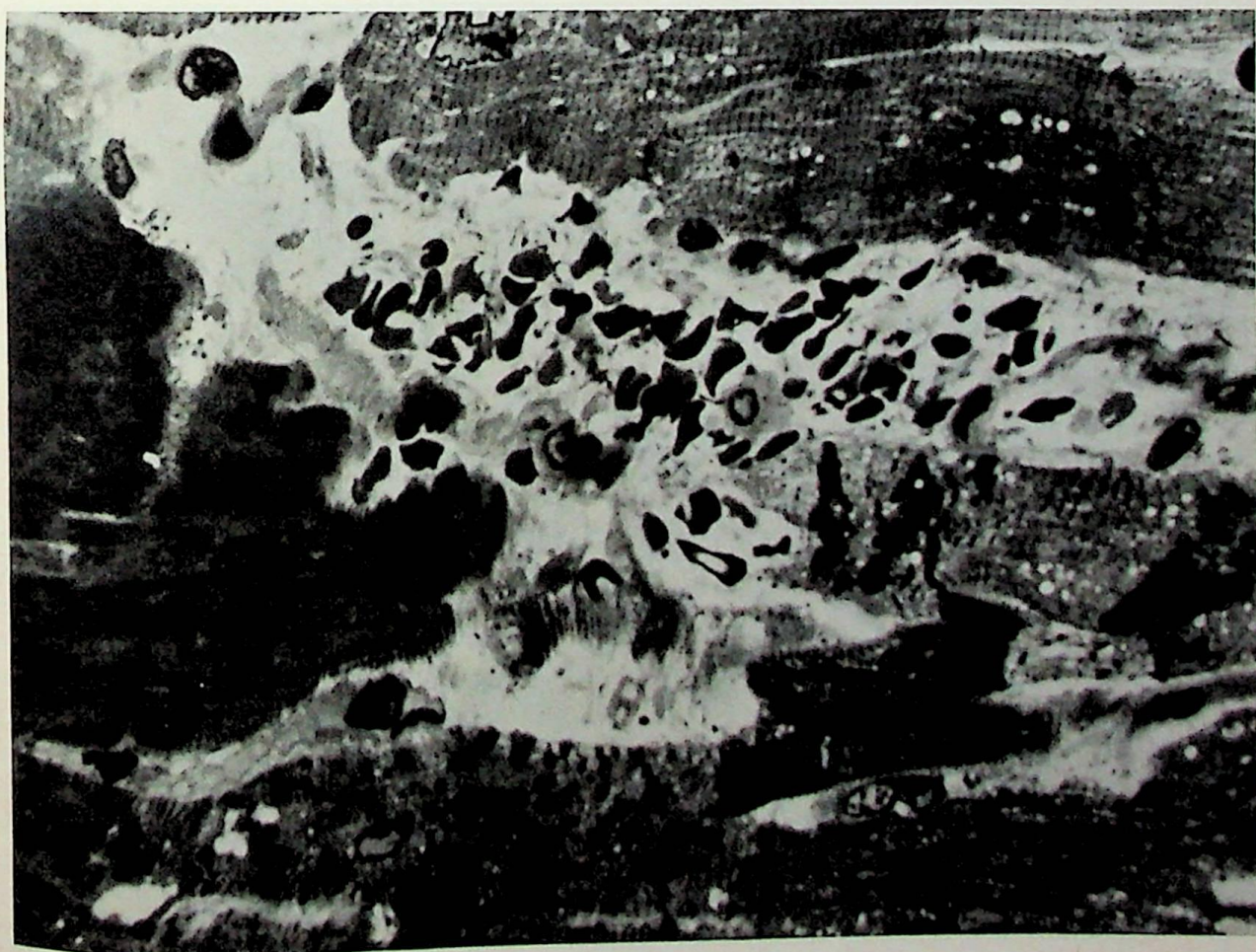
Fig. 7. Photomicrograph of focal hemorrhagic necrosis. Note extravasated erythrocytes surrounded by hypertrophied muscle fibers with contraction bands. (Azure II and methylene blue; ×1,000.)
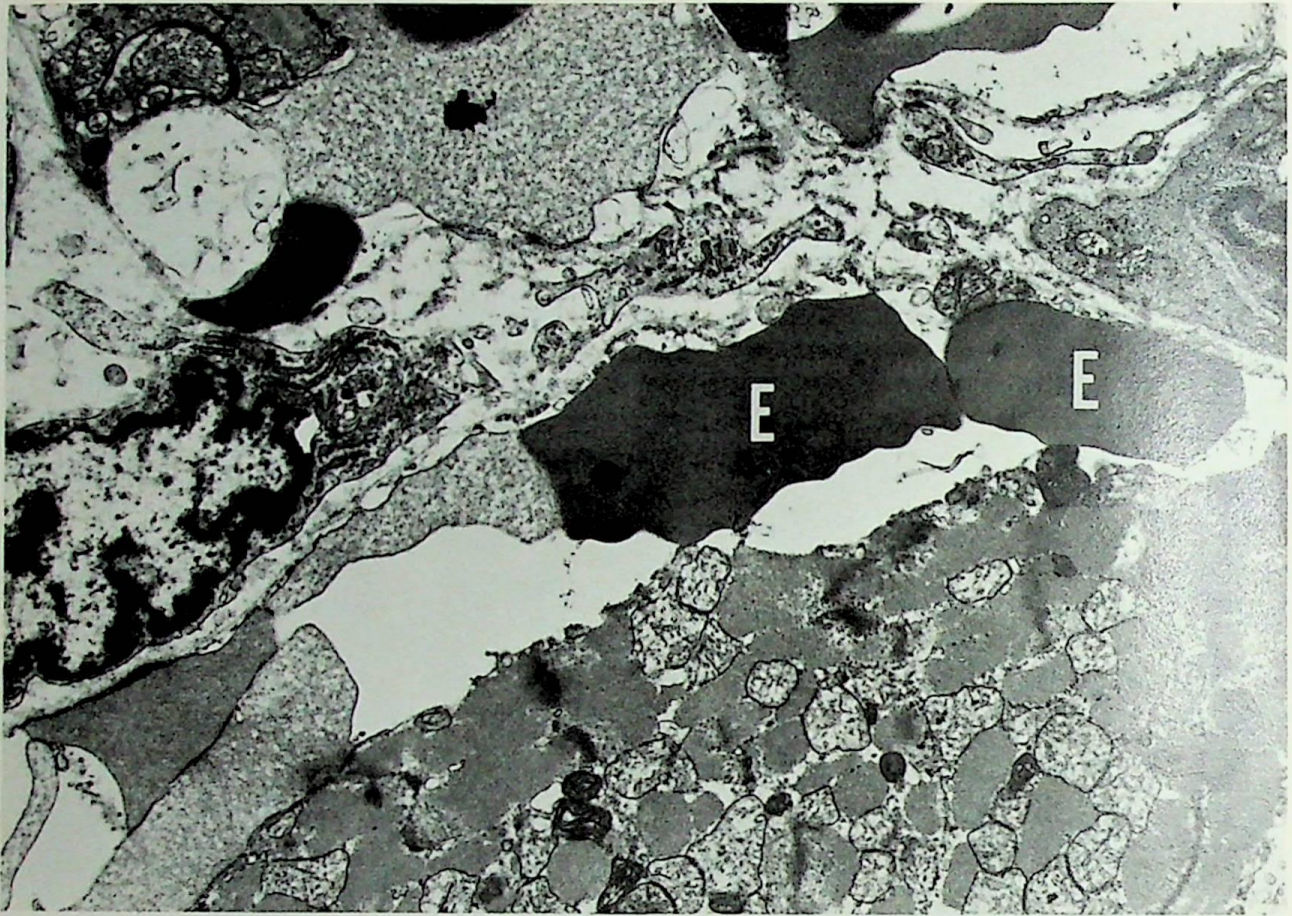
Fig. 8. Electron micrograph of stone heart. Note extravasated erythrocytes (*E*) in subsarcolemmal space of ischemic myocardial fiber. (Uranyl acetate and lead citrate; ×15,500.)
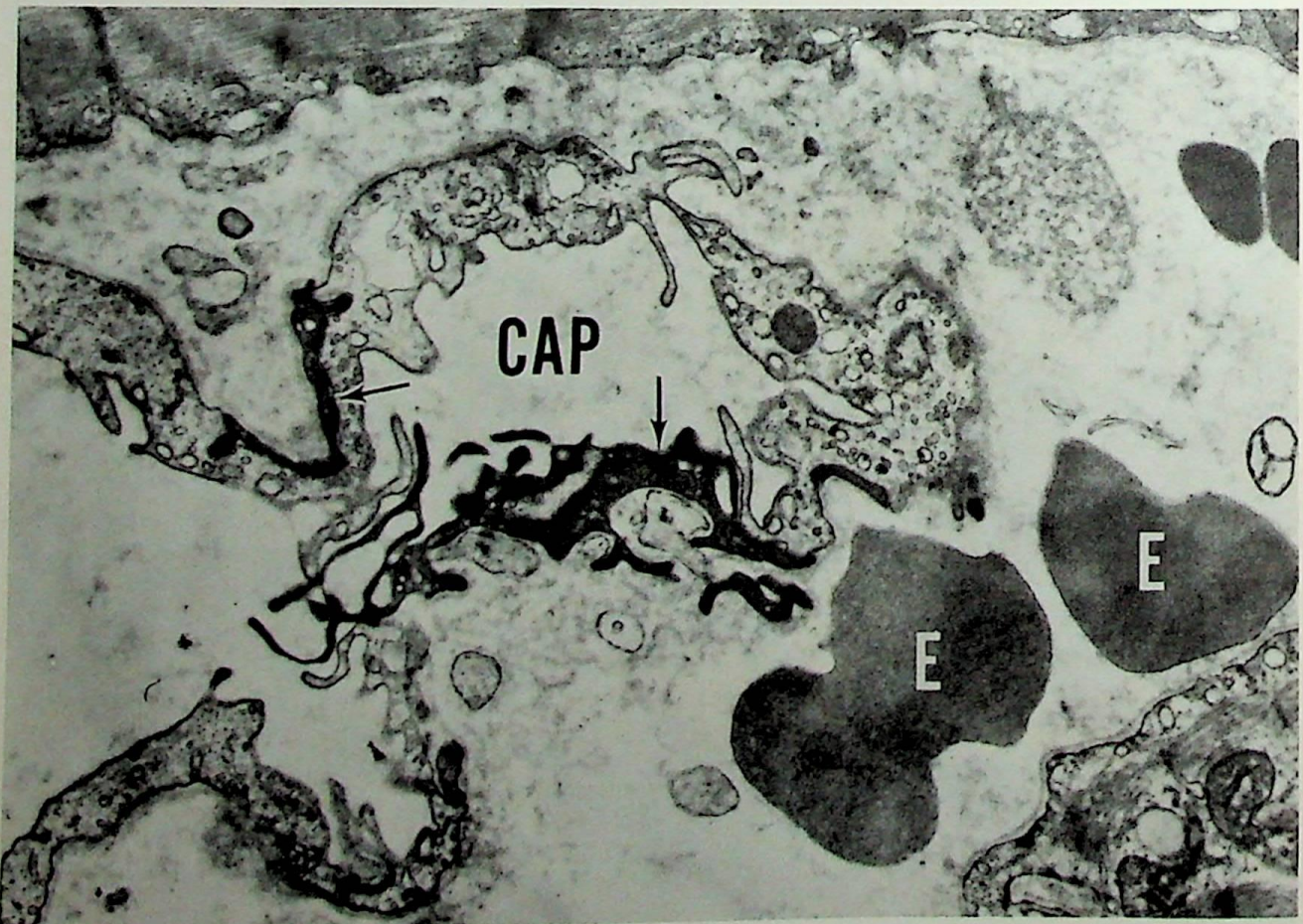
Fig. 9. Photomicrograph of myocardial capillary (*CAP*) with signs of anoxic injury. Note dark attenuated endothelial cells (*arrows*) wedged in between swollen endothelial cells with prominent cytoplasmic vesicles, and escaped erythrocytes (*E*). (Uranyl acetate and lead citrate; ×15,500.)
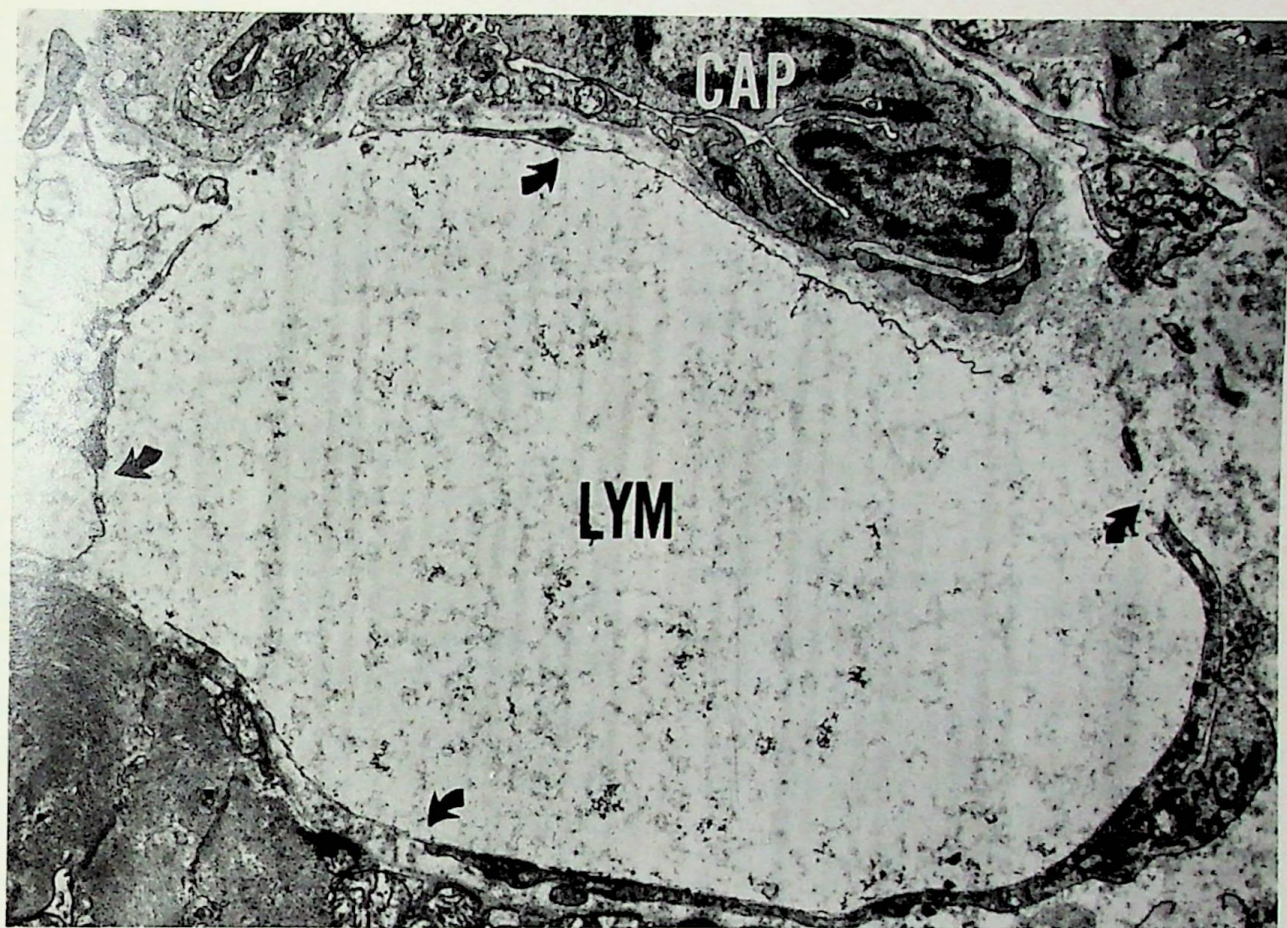
Fig. 10. Photomicrograph of markedly dilated myocardial lymphatic (*LYM*) next to capillary (*CAP*) with swollen endothelial cells. Note breaks in lining of lymphatic (*arrows*). (Uranyl acetate and lead citrate; ×12,400.)

contraction; a supercontracted fiber lay alongside a relaxed fiber or was separated from an overstretched fiber by a "torn" intercalated disk (Fig. 3 and 4). The contraction bands represented closely approximated Z-lines of the shortened sarcomeres, not unlike the collapsed ribs of an accordion. Intracellular edema was evidenced by the separation of myofibrils and subsarcolemmal blebs.

Mitochondrial Degeneration and Autodigestion.—The most frequently observed mitochondrial changes were bizarre configurations and vacuolation of the cristae matrix. Electron-dense inclusion bodies were seldom present. The Gomori technique for demonstrating hydrolytic enzymes showed a loss of primary lysosomes and markedly reduced acid phosphatase activity. The degenerated mitochondria were often in company with numerous large secondary lysosomal bodies in different stages of autodigestion. The autophagic lysosomes, some of which were membrane-bound, were filled with osmophilic debris apparently derived from incompletely digested organelles (Fig. 5). In rare instances, mitochondria in various stages of degradation and naked membrane structures were engulfed in a giant vacuole having a double-layered wall (Fig. 6).

The Microcirculation.—Interstitial hemorrhage was common in foci of myofibrillar degeneration, and extravasated red blood cells were abundant in the widened subsarcolemmal space (Fig. 7 and 8). The capillary endothelium often appeared completely interrupted in several places and showed remarkable structural alterations (Fig. 8 and 9). Some capillary endothelial cells became attenuated, with markedly increased cytoplasmic density, while others were swollen and contained large intracellular vesicles. The sharp contour of the basement membrane of the normal capillary wall also became indistinct (Fig. 9). Lymphedema was a prominent finding. At low magnification, the myocardial biopsies showed widened extracellular spaces that were filled with low-density amorphous proteinaceous material. Dilated lymphatics were lined by attenuated endothelial cells that were discontinuous (Fig. 10).

DISCUSSION

The salient clinical and anatomic characteristics of stone heart have been described previously.[2,3,7] Our present ultrastructural study indicates that a series of striking degenerative processes involving the contractile elements, the mitochondria, and the micro-

circulation occur when a vulnerable (hypertrophied) heart undergoes irreversible ischemic contracture.

Myofibrillar degeneration or myocytolysis (Fig. 1 through 4) is a common form of cardiac muscle injury. It has been observed in a variety of human and experimental conditions, including anoxia, oxygen inhalation, hemorrhagic shock, electric shock, potassium deficiency, cobalt toxicity, and administration of sympathomimetic amines.[9] It also occurs often after open-heart surgery with total cardiopulmonary bypass.[10] An earlier study by Gott and associates[1] and the more recent experience of Reul and associates[11] and MacGregor and associates[6] suggest hypothermia as a possible means of either preventing or reversing myocardial rigor. But strangely enough, myofibrillar degeneration has also been observed as a form of hypothermal injury.[12]

Reichenbach and Benditt[9] suggest that myofibrillar degeneration of different origins may depend on a common pathogenetic mechanism—namely, catecholamines released locally from sympathetic nerve endings in the myocardium or from the adrenal glands, or both. Catecholamines increase myocardial oxygen consumption by increasing the work of the heart and by uncoupling oxidative phosphorylation.[13] Either or both of these cellular mechanisms could cause a diminution in ATP levels necessary for maintenance of membrane integrity, ionic fluxes, and contractile phenomena of myocardial fibers.

Mitochondrial degeneration (Fig. 5 and 6) in stone heart, characterized by swelling, vacuolation, and fragmentation or loss of cristae, is similar to that observed in experimental ischemic arrest of normal and failing dog hearts.[4,6,14-16] However, the deposition of intramitochondrial electron-dense (calcium) granules, so commonly seen in the classic myocardial ischemic injury, is an infrequent finding. The presence of autophagic vacuoles and decreased acid phosphatase activity may represent a preexisting lysosomal degradation of mitochondria in the hypertrophied hearts. Autodigestion by the coalescence of mitochondria with lysosomes has been observed in aging rat hearts,[17] diseased human hearts,[18] and dog hearts after cardiopulmonary bypass.[16] The role of the lysosomal system in ischemic injury to the myocardium recently has been receiving greater attention.[16,19,20]

Structural alteration of the microcirculation in stone heart also reflects the ischemic basis of myocardial rigor. Intracellular edema has long been known to be an early sign of tissue response to anoxia,[21] and we have further demonstrated that lymphedema is also a prominent feature (Fig. 7 through 10). Edema and necrosis of capillary endothelial cells, coupled with lymphangiectasis, increase the vulnerability of the compromised heart and accelerate the processes of myocardial organelle degeneration. In experimental animals, ischemic injury to the myocardium after coronary artery ligation can be augmented by the obstruction of the cardiac lymphatics.[22] Focal hemorrhagic necrosis (Fig. 7 and 8) occurs more commonly in experimental acute myocardial infarction with concurrent lymphatic obstruction.

The occurrence of myocardial rigor has been attributed to derangement of the usual spatial relationship between the actin and myosin myofilaments because of low concentrations of ATP.[2,23] Our investigation indicates that the ultrastructure of tissue in stone heart reflects a state of global anoxia. Anaerobic glycolysis generates a limited supply of ATP, but as an energy provider glycolysis is grossly inadequate compared with oxidative phosphorylation. The overloaded hypertrophied hearts are more susceptible to the development of ischemic contracture because they have been functioning precariously with severely depleted stores of high-energy phosphate compounds. About 50% of the ATP reserve may be lost when the weight of the human heart exceeds 500 g.[24]

It may be unnecessary to invoke excessive calcium binding of the troponin-tropomyocin complex (the protein regulator of muscle contraction and relaxation) as a biochemical mechanism of myocardial rigor.[2] Our ultrastructural studies show, albeit indirectly, no accumulation of calcium in the organelles. Muscle tension develops when the concentration of magnesium-ATP falls below a critical level, independent of the presence or absence of calcium ions.[25] When myosin is not bound to magnesium-ATP, interaction with actin cannot be blocked by troponin-tropomyosin. Bonds known as "rigor complexes" develop, regardless of calcium ion concentration, and contracture occurs.[26]

The metabolic response to ischemia of any individual heart, normal or diseased, varies considerably. Clinically, stone heart has been observed to occur after 22 to 110 minutes of ischemia.[2,3] Even under the more rigidly controlled experimental situation, myocardial rigor can be produced after 38 to 70 minutes of ischemia.[6] Although myofibrillar degeneration, the morphologic characteristic of stone heart, is a nonspecific form of cardiac response to a variety of injuries, it might be causally related to the release of endogenous catecholamines.[9] Norepinephrine, the endogenous catecholamine of the mammalian heart, is released from sympathetic nerve endings at the onset of ischemic arrest. Increase in glycogenolysis and

glycolysis results from beta-adrenergic receptor stimulation by norepinephrine. The protection afforded by propranolol, a beta-adrenergic receptor blocker, in both clinical and experimental situations,[6,11] lends support to the theory that catecholamine plays a role in the pathogenesis of stone heart.

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Mar. 11 and 12	**Gynecologic Surgery**
Mar. 11 to 13	**Council on Medical Education of American Medical Association**
Mar. 18	**Pediatric Day**

For further information about the meetings, please write to Mr. William Nietz, Communications, Mayo Clinic, Rochester, MN 55901.

The First Fifty Years of *Mayo Clinic Proceedings*

JAMES T. PRIESTLEY, M.D.
EMERITUS STAFF

No one knew how long the new venture would survive. This was common in an institution where innovations were frequent. Successful ones were continued, modified, and improved. Others were dropped.

The *Bulletin of the Mayo Clinic and Mayo Foundation* appeared initially on Apr. 21, 1926. Within months the name was changed three times and ultimately it became *Proceedings of the Staff Meetings of the Mayo Clinic,* which title was retained until 1964. By then, the original fledgling publication had progressed through childhood and adolescence and was attaining maturity. What was its origin? What led to its continuing growth and recognition?

Starting before 1910, staff members of the Mayo Clinic and fellows of the Mayo Foundation met every Wednesday evening unless it was December 25, January 1, or July 4. A pathology conference* preceded staff meeting. Programs of staff meetings consisted mainly of clinical and investigative studies, but an important component was the reporting of meetings attended and visits to other medical institutions in the United States and abroad, and also yearly reporting from various departments. For surgical fields, these contained information on numbers and types of operations performed, advances in knowledge, changes in techniques, mortality rates, and other pertinent data. No one missed staff meeting if he was able to walk. Drs W. J. and C. H. Mayo always sat in the front row, which did not alleviate the nervousness of younger members as they rose to speak. In 1926, it was decided that presentations made at these weekly meetings should be published "for information of the staff of Mayo Clinic and fellows of Mayo Foundation for medical education and research." Thus was born what is now known as *Mayo Clinic Proceedings*.

Publication was weekly. Contents, although originally derived solely from staff meetings, soon included talks by prominent visiting physicians and surgeons as well as Mayo Foundation Lectures. There was no editorial board. Mrs. Maude Mellish,† whose background was that of medical librarian and editorial worker, was the first editor. In addition to the staff of Mayo Clinic and fellows of Mayo Foundation, "The Proceedings" was sent free of charge to any physician, medical student, or library that requested it. There was no advertising. The total cost was borne by Mayo Clinic. From 1931 to 1942 there were about 16 pages per issue.

One hesitates to mention outstanding contributions that brought growing recognition to the journal during these early years, as so many would have to be omitted. If a selection was made, hopefully without

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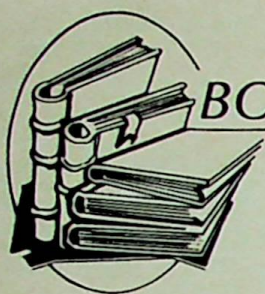
*This conference was conducted by an uninhibited pathologist who could present findings on any patient who had died during the previous week. The responsible physician or surgeon was then called on to explain and, if need be, defend his diagnosis and treatment. This was a "no holds barred" conference.

†Later she became the wife of Dr. L. B. Wilson, first director of Mayo Foundation.

giving offense to those not included, it might comprise: a wide variety of articles by W. J. and C. H. Mayo; reports on the biliary and gastrointestinal tracts by E. S. Judd, D. C. Balfour, W. Walters, and G. B. Eusterman; thoracic surgery by S. W. Harrington; neurologic surgery by A. W. Adson; diabetes by R. M. Wilder; renal function and disease by N. M. Keith and L. G. Rowntree; urography and transurethral surgery by W. F. Braasch, H. C. Bumpus, and G. J. Thompson; physiology of the liver and pathologic physiology of peptic ulceration by F. C. Mann and J. L. Bollman, as well as other physiologic studies by W. C. Alvarez, H. E. Essex, and C. F. Code. Later appeared the highly significant work of W. H. Feldman and H. C. Hinshaw on chemotherapy for tuberculosis and that of E. C. Kendall and P. S. Hench on cortisone.

Changes were made as years passed and circulation increased. Mrs. Eleonore Clappier became managing editor in 1942, a position she filled with dedication and distinction until her retirement in 1975. Gradually, fewer articles presented at staff meetings were published and, conversely, more articles from other sources were included. By 1960, only half of the articles published had been presented at staff meetings. Selected abstracts were added. The base was being broadened. Weekly publication was continued from 1926 until 1942 when, because of World War II, publication became bimonthly. By 1955, the mailing list exceeded 30,000.

Discussions starting in 1962 led to significant changes in policy in 1964. These were directed toward converting the journal into the recognized pattern of national medical journals. The name was changed to *Mayo Clinic Proceedings*. An editorial board of six members, with Dr. E. D. Bayrd as editor-in-chief, was established in 1964. Henceforth all articles submitted had to be acceptable to this board. By this time, the nature of staff meetings had changed and little material from these meetings was used. Original articles were submitted by residents and staff. Publication became monthly and the number of pages per issue was increased, ranging from 66 to 80. Circulation now exceeded 48,000. Expenses were rising so, in January 1971, advertising was accepted for the first time and the format was redesigned. Names, including medical students, interns, and residents, were still added to the mailing list without charge but a footnote now indicated that those who wished could send $7.00 yearly to Mayo Foundation (recently increased to $12.00). Twenty percent of those on the list were canvassed each year and those who did not indicate a definite desire to be retained were dropped. Contents now included clinicopathologic conferences, subject and book reviews, abstracts, medical history vignettes, and notices, in addition to original articles. Starting in 1971, Dr. A. B. Hayles became editor-in-chief. Dr. C. G. Roland was named executive editor in 1972. Mrs. Donabeth C. Postier was appointed managing editor in 1975. Circulation was then 65,000, 27% of which went to other countries. Current circulation is exceeded by only three or four medical journals on this continent (excluding controlled circulation magazines). A recent analysis revealed that internists made up the largest number on the mailing list, followed in descending order by surgeons, family physicians, pathologists, and pediatricians. Ninety-five of 132 articles submitted in 1975 were published.

So, from a modest beginning, solely as a report of staff meetings, *Mayo Clinic Proceedings* is now a diversified medical journal of national and international distribution and recognition. Contributions by staff and residents of Mayo Clinic and Mayo Foundation remain the major but not the only source of its contents. Its growth and development over a period of 50 years attest to its reception in the medical world. I believe that Dr. Will and Dr. Charlie would wish to join many others in expressing appreciation and congratulations to all who have contributed to an achievement far beyond any original expectations.

Of Acceptable Risk (Science and the Determination of Safety), by William W. Lowrance, 191 pp, with illus, $4.95, Los Altos, CA: William Kaufmann, 1976

There is an irony common to many educational experiences wherein the most stimulating lecture or the most helpful review appears to tell us nothing that is new. When the logic is solid, the presentation of information clear, and the pace appropriate we often believe we know it all, even as it unfolds. I felt this way about the present book.

Lowrance is a chemist who wrote the book whilst Resident Fellow of the National Academy of Sciences (1973 to 1975), under the general guidance of a blue ribbon committee comprising the Panel on Science and the Determination of Safety. With resolute objectivity, he addresses the questions, by whom, by what means, and when should judgments be made as to what is safe and what is not? His definition: "A thing is safe if its attendant risks are judged to be acceptable," The scope is broad—foods and drugs, occupational hazards, ionizing radiation, health risks from manufactured goods, and ecologic consequences of waste are all considered. These issues clearly bring up questions of fact and questions of value judgment; the two are separated clearly and logically. Important but distracting social aspects such as personal or corporate carelessness, selfishness, and dishonesty are deliberately avoided.

After the Introduction, Chapter 2 discusses measuring risk. The scientific method is described with beautiful simplicity; statistics, epidemiology, occupational hazards, and problems in human experimentation are discussed. Chapter 3 shifts emphasis to the value aspects and is entitled "Judging Safety." Chapters 4 and 5 examine the logistics and inherent problems of legislative, executive, and judicial aspects of safety. Chapter 6 is a detailed review of the DDT story, an excellent example that summarizes all that has been said.

A special point concerns the ambivalent role of the scientist, as both expert witness and member of society. Lowrance quotes from Eugene Rabinowitch: "Scientists . . . and physicians should render their conclusions without presumption as to what point of view they are to defend. If . . . their conclusions begin to be affected by extra-scientific reasons . . . they must have intellectual honesty [and state that] from here on I will speak also as an . . . ideologically committed citizen. . . ."

This book gives us a lead toward the objectivity needed for such an approach. However, it offers even more to the nonexpert by giving in simple style both the data and dilemmas and the scientific and moral issues that must be considered before society can answer these complex questions. The author aims for a "diverse readership." I believe this could well extend to all responsible members of society and would like to see something like this book listed as required reading for all high school seniors.

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Division of Gastroenterology and Internal Medicine

The Kidney, Volumes I & II, edited by Barry M. Brenner and Floyd C. Rector, Jr., 2,064 pp, with illus, $85, Philadelphia: W. B. Saunders Company, 1976

This multiauthored textbook of nephrology succeeds in presenting comprehensive reviews of the basic sciences that underpin nephrology and of the pathogenesis and pathophysiologic consequences of renal diseases and their management. Most of the authors have produced timely, extensively referenced chapters, some of which will become classics.

A few authors have failed to present a balanced view, relying too heavily on their own personal investigative results and underemphasizing the equally compelling data or viewpoints of other investigators. In a less scholarly textbook this would not be a valid criticism. In this book it is a disappointment.

Although it is not easy to read, the book is worth studying. The effort involved in reading derives mainly from the decision to make each chapter a thorough review of the literature. The reader may occasionally be distracted by lines where more space is devoted to numerical referencing than to text. The student is rewarded by the high quality of reference articles cited. This is a valuable source of reference material not otherwise available in collected form.

There is something for everyone in this textbook. It has to appeal to professionals of highly varied interests and backgrounds, so many will find some chapters too complex or not consonant with their interests; these may be easily left for another time (or be unread).

When one applies the test of relevance to practice in the hospital setting, the book stands up well. In some areas, science is overstressed at the expense of helpful clinical information but this could be corrected in succeeding editions.

Among my colleagues, various chapters have their champions, but there is widespread agreement that in each section there are superior examples of the art. The editors have done a marvelous job in bringing forth such a collection of chapters, which stand well alone and yet are so well cross-referenced.

On balance, this textbook of nephrology has no current equal. Adequate review will require comparison with the forthcoming third edition of Strauss and Welt's *Diseases of the Kidney* (Little, Brown & Company). In the meantime, and perhaps even after, Brenner and Rector's *The Kidney* must be regarded as the best reference textbook available in nephrology.

Cameron G. Strong, M.D.
Division of Nephrology and Internal Medicine

Non-invasive Cardiac Diagnosis, by Edward K. Chung, 331 pp, with illus, $18, Philadelphia: Lea & Febiger, 1976

This text adequately achieves its primary goal of describing common noninvasive cardiac diagnostic problems frequently encountered in daily practice. Sixteen chapters cover a wide variety of noninvasive topics including discussions of electrovectorcardiography, exercise electrocardiographic stress testing, computer electrocardiography, echocardiography, phonocardiography, systolic time intervals, and drug level determinations. Eighteen contributors have provided a wide variety of expertise.

At its onset, the editor indicates that this text is intended to be clinical, concise, and practical to assist all physicians directly in their daily care of patients with common cardiac problems. Despite some

of the problems of touching lightly on a wide variety of noninvasive subjects, this text certainly achieves much of its goal by providing concise clinically oriented discussions. Many areas, such as noninvasive roentgenographic studies, phonocardiography, and systolic time intervals, are thoroughly and adequately covered. Because of their rapid expansion, other areas such as echocardiography are represented by only a preliminary and incomplete discussion.

One subject which, unfortunately, is neglected significantly is that of congenital heart disease—which is provided only brief and cursory review under echocardiography. Most of the references provided are relatively recent and extend through 1974 with a few from 1975. These are certainly adequate to suffice for most of the topics presented.

Overall, this text represents an excellent primer for the student in cardiovascular diseases and for practicing physicians. It offers an excellent and concise review of commonly used noninvasive diagnostic methods for cardiac diseases.

Donald J. Hagler, M.D.
Division of Pediatric Cardiology

The Illustrated Manual of Sex Therapy, by Helen Singer Kaplan, 193 pp, with illus, $14.95, New York: Quadrangle/The New York Times Book Company, 1975

"For proper sexual functioning to occur a person must not only be free of intense negative affect but must also be free of excessive cognitive control. . . . This is also true of other biological functions which are controlled by the autonomic nervous system. Digestion, respiration and cardiovascular functions are similarly innervated by the visceral nerves and are similarly subject to impairment by emotional and cognitive factors." Kaplan made this point compellingly in *The New Sex Therapy* (Quadrangle, 1974). Considering sexual dysfunction as a true psychophysiologic disorder, she gave scientific basis for a flexible, multifaceted treatment program—educational, psychotherapeutic, marital, and experiential.

This beautiful volume supplements *The New Sex Therapy*. It is intended to help practitioners, teachers, students of medicine and psychology, and patients themselves to understand and overcome obstacles to full sexual function. Six main sexual disabilities are discussed and their treatment is described and illustrated: frigidity, female orgasmic dysfunction, vaginismus, impotence, retarded ejaculation, and premature ejaculation. Kaplan is precise and specific about "how we do it" at the Payne Whitney Clinic—even to the point of "how to ask it" and "how to say it." The beautiful line drawings by David Passalaqua artistically add the "show" to the "tell." The result is a valuable handbook for the consulting room of any sex therapist.

In a closing chapter, the author is "uneasy" that her presentation may have been "too mechanical." I do not believe this is the case. Kaplan alludes repeatedly and persistently to intrapsychic and dyadic factors that influence sexual behavior, and the reader has no doubt that psychodynamics can be critically important. However, the "mechanical" nature of the book is its greatest value for psychotherapists who can "cure" conflicts competently yet remain impotent when faced with their patients' sexual disabilities. "How to do it" is sufficient and praiseworthy in any manual. For the reader who needs and wants more, Helen Singer Kaplan's previous publication will serve admirably.

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Department of Psychiatry
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Electrocardiographic Excursions: Vulgaria et Exotica, by Leo Schamroth, 170 pp, with illus, $31, Oxford: Blackwell Scientific Publications, 1976

Schamroth has not written a primer on basic electrocardiography but rather a book specifically designed to focus on essential features of electrocardiography. The text is cleverly laid out with clear succinct illustrations for each electrocardiographic abnormality displayed. Adequate references are supplied to support the narrative.

For anyone who has been a follower and fan of Sherlock Holmes, the book has considerable value as deductive entertainment. The simplicity of the text coupled with its entertaining presentation makes for easy reading. Unlike most textbooks on electrocardiography, this one left me with a feeling of enjoyment after having read through it.

The textbook has limited appeal for those looking for detailed basic understanding of electrocardiographic physiology and certainly should not be considered a mandatory textbook for one's personal library of cardiology reading. But for the student of electrocardiography who wishes to spend a few happy hours reviewing the subject in a lighter vein, this text has many specific pointers, clearly and entertainingly presented. The brevity of the text does not allow for extended study of any one subject.

I believe that the author, in dedicating his book to Sherlock Holmes, has created a pleasing work that lives in the tradition of A. Conan Doyle's works. Inspection, appreciation of detail, and deductive reasoning—the tools of Sherlock Holmes—are applied here with pleasing finesse to the 50 ECG problems presented. The quotation included by the author and attributed to Holmes perhaps best summarizes the essence of the message in this book: "How often have I said to you, that when you have eliminated the impossible, whatever remains, however improbable, must be the truth." We can almost hear the echoed added statement, "It's elementary, my dear Watson." Electrocardiography has been enhanced by this well-written text and my congratulations go to its author.

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Medicine

Clinical Implications of Air Pollution Research, edited by Asher J. Finkel and Ward C. Duel, 411 pp, with illus, $20, Acton, MA: Publishing Sciences Group, 1976

The chapters of this careful review of the literature are written by well-established, seasoned clinicians who have screened some of the major contributions to the field. The book is divided into five parts, covering respiratory diseases, cardiovascular diseases, immunology (including hypersensitivity and host defense), the central nervous system and sense organs, and illnesses of children.

In part one, Shy reviews the epidemiologic evidence for the incidence of lung cancer in the urban environment. He sites demographic data related to population density, smoking habits, urban and rural types of pollution, and the prevalence and incidence of lung cancer. There is a section devoted to the changes in respiratory epithelium induced by BENZO (a) PYRENE, especially metaplasia. Selikoff and Shubik discuss the role of particulate air pollution in lung cancer. The remainder of this section is devoted to discussions on the chemical and physical properties of particular pollutants, the need for and the implications of further research in air pollution, and respiratory diseases. One leaves this section with more appreciation of the complexity of interactions between pollutants, their wide variety, and biologic variations observed within the population.

Although the section on cardiovascular diseases covers many of the key issues in air pollution, it is not all-inclusive. It tends to focus on the role of carbon monoxide with respect to angina pectoris, intermittent claudication, hemodynamics, and the performance of cardiac patients under

the influence of various loads of carbon monoxide. To the book's credit, the little-discussed effect of carbon monoxide on the susceptibility of the heart to arrhythmias such as fibrillation is reviewed by De Bias. These discussions are summarized and placed in proper perspective by the participants, especially Boyle, at the end of this section. It is regrettable that this section did not devote some space to reviews of the impact of sulfur dioxide, the oxides of nitrogen, and the nonspecific stresses of irritants upon the cardiovascular system.

In part three, the ever-burgeoning collection of information in the medical literature related to immune mechanisms and pulmonary diseases is addressed. The intimate biochemical composition of the lung and the properties of air pollutants are reviewed by Greene, whose obvious understanding of this field is noteworthy. The bronchospastic reaction is reviewed in an attempt to clarify relationships between various forms of air pollution and adults and children, from studies in Japan. A considerable case for the allergic mechanisms was made by Meyer. The relations of host defenses altered by pollutants to susceptibility to infectious diseases caused by viruses, bacteria, and fungi are explored.

In part four, the impact of air pollutants on the central nervous system and sense organs is discussed. Unfortunately, the time-lag between the effects produced by low levels of some air pollutants makes progress in this area difficult. It is always a problem to decide whether one is observing the accumulated damage to the nervous system or a progressive increase in body burden of the toxicant, as in the case when lead produces chronic low-level intoxication.

The fifth section is devoted to children. As most toxicologists know, the response of children often differs from that of adults. Children are usually more susceptible. The pollutants considered are particulate matter and sulfur oxides. Note is taken of the peculiar difficulties encountered in mass surveillance of the pediatric age group and the fact that data obtained by different pulmonary function techniques are not directly comparable.

Two special contributions are noteworthy. One is written by Todd and relates to man's efforts at environmental control, especially those made in the United States under the Clean Air Act and similar statutes, and the competing interests whose economic gains may be in jeopardy. The other special contribution is by Congressman Brown, in which he describes air pollution as a social disease. Brown discusses legislation, voluntary control, the economic impacts of air pollution, the constraints imposed by interest groups, and the controversies that result.

Certainly, the impact of air pollution touches more than the human population if it involves the flora and the fauna that enter our food chain. It might have been helpful had some discussion been given to this aspect. So little is known about the effects of air pollution on pregnancy that one wishes that what data are available had been mentioned. Our society certainly needs further studies of this nature. Several times in this volume reference was made to pollutant interactions and their health sequelae. This volume is laudable and serves as a good source for certain key studies.

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Fundamentals of Urology, by Jack Lapides, 609 pp, with illus, $17.50, Philadelphia: W. B. Saunders Company, 1976

Nesbit's *Fundamentals of Urology* has been a favorite of urologic students and residents for many years. This current edition by Lapides and 22 coauthors again provides an excellent current source of urologic core material for the developing urologist. The variety of subjects should also make this text an excellent reference for the nephrologist, general surgeon, or family physician—indeed, for anyone seeking information on urologic diseases.

From the initial and incomparable review of genitourinary anatomy, the subjects are handled in a fundamental and complete fashion so that the topics are understandable to the neophyte as well as to the advanced student. Genitourinary pathology, a topic often omitted or inadequately presented in other urologic texts, is thoroughly covered in two chapters. This provides an excellent reference for the pathophysiology of benign and malignant diseases affecting the urinary tract.

Basic to all urology is the physiology of micturition. Many of the current concepts of urodynamics and the process of micturition originated through work done at the University of Michigan, and these topics are thoroughly covered in the text. Additionally, abnormalities of micturition resulting from neurologic or metabolic disease are thoroughly presented, depicting their resultant urodynamic profiles. With an understanding of these urodynamic changes, the authors present current treatment programs for each abnormality.

As the authors have stated, the book is intended mainly for the developing student of urology; thus the remainder of the chapters deal with the individual maladies of urologic practice. Each topic covers thoroughly the etiologic agent for the disease process and the various treatment modalities available. A fine chapter on urinary tract infections is presented and the concept of host resistance is explained; this concept is the basis for current urologic management of factors leading to urinary tract infection and, as with many concepts of urodynamics, was also an original contribution of the authors of this chapter.

It has been a pleasure to review this welcome addition to the literary armamentarium of the urologic student. For the price asked, the book contains a vast quantity of fundamental knowledge. It certainly should be a valuable text to anyone who desires a lucid, concisely presented volume of urologic core material.

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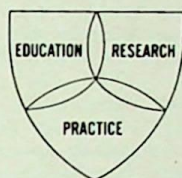
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